The effects of Cannabidiol oil derived from Hemp on the growth performance and wellbeing of *Oreochromis niloticus*

by

Timothy Craig Juries

Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science

at

Stellenbosch University
Department of Animal Sciences, Faculty of AgriSciences

Supervisor: Dr Khalid Salie
Co-supervisor: Dr Helet Lambrechts

December 2021

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2021

Summary

Cannabis sativa is an herbaceous plant that contains a cannabinoid known as Cannabidiol (CBD) which has similar properties as delta-9-tetrahydrocannabinol (THC), without the psychoactive effect. Industrialised intensive production systems are becoming more prevalent in the aquaculture industry, which indirectly increase the pressure on fish resources due to the inclusion of fish meal in fish diets. Alternative protein feeds for the replacement of fishmeal are available, but diet palatability is compromised when alternative protein sources are used. The complex endocannabinoid system is widely distributed throughout the body and play an essential role in modulatory functions like immune status, inflammation, and emotional responses. *Cannabis sativa* contains more than 150 phytocannabinoids which can be found in resin within flowers, leaves, and seeds. Cannabidiol (CBD) is the second most studied cannabinoid to THC. Cannabidiol is an antagonist at the CB1 receptor and an agonist at the CB2 receptor which has an immunomodulatory effect that stimulates the immune system to fight disease as well as an anxiolytic effect in humans.

In this study, the potential effect of CBD oil, as a feed additive, was studied in Nile tilapia (*Oreochromis niloticus*, 1758) through monitoring of growth (final body weight and body length) and haematological parameters (i.e. white blood cells, red blood cells, and platelets). Four treatments were prepared with increasing CBD concentrations (i.e. 0 mg/kg, 20 mg/kg, 40 mg/kg, and 60 mg/kg). The treatment diets were fed to Nile Tilapia for a duration of 10 weeks at 4% body weight, four times a day. Data recorded fortnightly included live weight, length, overall survival, and feed conversion ratio (FCR). Blood samples were collected fortnightly, and flow cytometry was used to perform a blood cell count.

The respective CBD diets did not influence growth parameters, specific growth rate (SGR), and FCR. Water quality parameters (i.e. water temperature, pH and dissolved oxygen (DO)) recorded were all in the optimum range for *O. niloticus*. A significant difference for Fulton's condition index was reported among the treatments, indicating that increasing CBD inclusion resulted in leaner fish. The different CBD diets did not affect blood cell counts, indicating that CBD inclusion did not affect fish wellbeing in this study. The results obtained in this study did not conclusively indicate a treatment effect that recommend the use of CBD as an additive in fish diets for improved growth and overall fish wellbeing.

Opsomming

Cannabis sativa is 'n kruidagtige plant wat 'n cannabinoïde bevat bekend as Cannabidiol (CBD) wat soortgelyke eienskappe het as delta-9-tetrahydrocannabinol (THC), sonder die psigo-aktiewe effek. Geïndustrialiseerde intensiewe produksiestelsels word meer algemeen in die akwakultuur bedryf, wat indirek die druk op visbronne verhoog as gevolg van die insluiting van vismeel in visdiëte. Alternatiewe proteïenvoere vir die vervanging van vismeel is beskikbaar, maar dieet smaaklikheid word benadeel wanneer alternatiewe proteïenbronne gebruik word. Die komplekse endokannabinoïde stelsel is wyd versprei deur die liggaam en speel 'n noodsaaklike rol in modulerende funksies soos immuunstatus, inflammasie en emosionele reaksies. Cannabis sativa bevat meer as 150 fitokannabinoïede wat in hars in blomme, blare en sade gevind kan word. Cannabidiol (CBD) is die tweede mees bestudeerde cannabinoïde teenoor THC. Cannabidiol is 'n antagonis by die CB1-reseptor en 'n agonis by die CB2-reseptor wat 'n immunomodulerende effek het wat die immuunstelsel stimuleer om siektes te beveg asook 'n anxiolitiese effek by mense.

In hierdie studie is die potensiële effek van CBD-olie, as 'n voeradditief, in Nyl-tilapia (Oreochromis niloticus, 1758) bestudeer deur monitering van groei (finale liggaamsgewig en liggaamslengte) en hematologiese parameters (dws witbloedselle, rooibloedselle en bloedplaatjies). Vier behandelings is voorberei met toenemende CBD-konsentrasies (d.i. 0 mg/kg, 20 mg/kg, 40 mg/kg en 60 mg/kg). Die behandelingsdiëte is vier keer per dag aan Nile Tilapia gevoer vir 'n duur van 10 weke teen 4% liggaamsgewig. Data wat tweeweekliks aangeteken is, het lewende gewig, lengte, algehele oorlewing en voeromsettingsverhouding (FCR) ingesluit. Bloedmonsters is tweeweekliks versamel, en vloeisitometrie is gebruik om 'n bloedseltelling uit te voer.

Die onderskeie CBD-diëte het nie groeiparameters, spesifieke groeitempo (SGR) en FCR beïnvloed nie. Waterkwaliteit parameters (d.i. watertemperatuur, pH en opgeloste suurstof (DO)) wat aangeteken is, was almal in die optimum reeks vir O. niloticus. 'n Beduidende verskil vir Fulton se toestand-indeks is onder die behandelings gerapporteer, wat aandui dat toenemende CBD-insluiting tot maerder visse gelei het. Die verskillende SSK-diëte het nie bloedseltellings beïnvloed nie, wat aandui dat SSK-insluiting nie viswelstand in hierdie studie beïnvloed het nie. Die resultate wat in hierdie studie verkry is, het nie beslis 'n behandelingseffek aangedui wat die gebruik van CBD as 'n bymiddel in visdiëte aanbeveel vir verbeterde groei en algehele viswelstand nie.

This thesis is dedicated to my late Grandmother, Mieta Langeveld and my mother, Valerie Juries.

‘Pieta’, Your spirit will surely live on in everything I do as well as your unspoken support. I am happy that I was able to make you proud.

To my mom, thank you for your unwavering support and your sacrificial care to ensure that I finish this work. Thank you for allowing me to follow my heart in everything I do.

To my aunt’s, Reona Juries and Tania Langeveld and my uncle, Jeremy Langeveld, thank you for the support throughout this journey from my first degree till my third, and to my Brother, Shane Juries and Cousins, Ronaldo, Robin and Tammy-Leigh, thank you for the interest and support.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- Dr Khalid Salie
- Dr Helet Lambrechts
- Mark Joscelyne
- Henk Stander
- Anvor Adams
- Dr Chris Fouche
- Shaun Kordom
- The Sustainable Agriculture support staff
- The Department of Animal Sciences
- The Department of Agronomy
- The Aquaculture Certificate Class of 2020
- Welgevallen Experimental Farm
- The National Research Foundation (NRF)
- Lastly, to everyone and all the institutions that indirectly assisted and supported me during a difficult Covid-19 research period

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at are those of the author and are not necessarily to be attributed to the NRF.

Outline

This thesis is presented as a compilation of 5 chapters.

Chapter 1 **General Introduction and project aims**

Chapter 2 **Literature review**

Chapter 3 **Growth Experiment**
Growth parameters for three treatments with CBD oil given at different concentrations

Chapter 4 **Haematology Experiment**
Effects of CBD oil extract on WBC, RBC and Platelets

Chapter 5 **General conclusion and recommendations**

Table of Contents

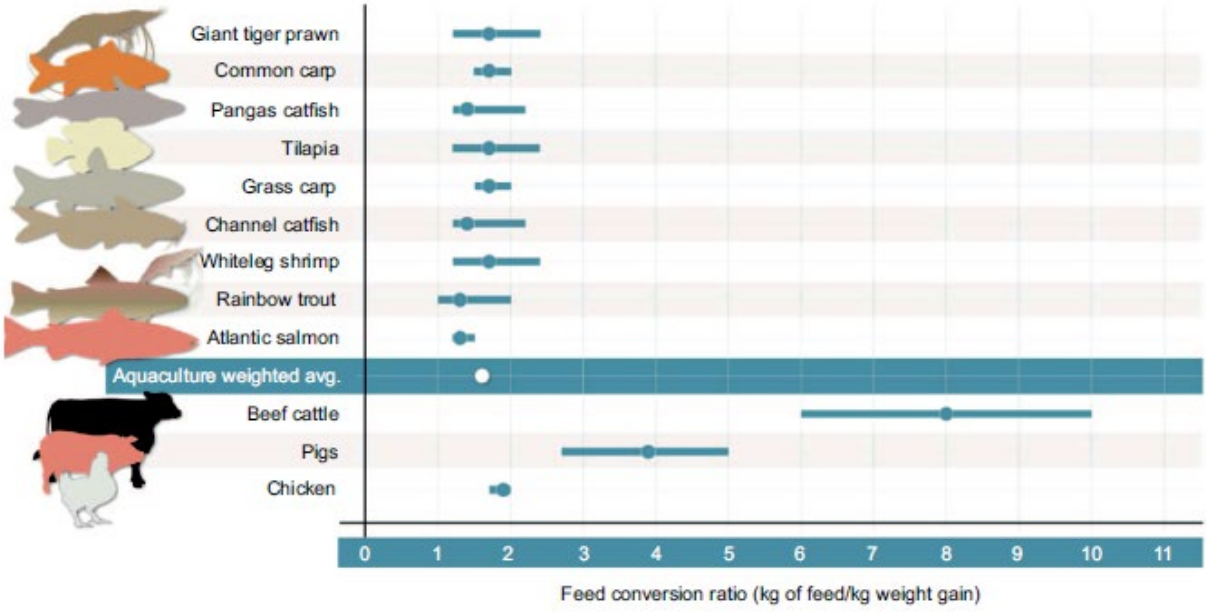
Table of Figures

Table of Tables

Chapter 1 General introduction and project aims

1.1. Introduction

Aquaculture is the fastest-growing food production sector in the world (Food and Agriculture Organization, 2020), however, the biggest hurdle in ensuring cost-efficient production is the price of fish feed, being as much as 60% of the production costs (El-Sayed, 2006). To put the size of the sector in context, in 2018 the aquaculture sector contributed about 82 million tonnes which equates to about 46% of the Global fish production, valued at USD 250 Billion (FAO, 2020). According to the FAO (2020), this contribution is set to increase to 60% around 2030. Apart from the food fish contribution, additionally the aquaculture sector produced 32.4 million tonnes of aquatic algae, and 26 000 tonnes of ornamental seashells and pearls (FAO, 2020).



One of the most expensive components in a fish diet is protein. The protein in any fish feed is required for growth, and the most common source of protein in fish diets is fish meal (FM). Tacon & Metian (2015) and Fry *et al.* (2018) suggest that aquaculture feed is characterized by a significant inclusion level of raw materials consisting of processed wild-caught fish. Madage *et al.* (2015) postulated that the practice of using FM is an unsustainable practice, and provides contradicting evidence that aquaculture is more sustainable than other livestock production systems.

Aquaculture nutrition research focus on the replacement of fish meal with alternative feed protein sources, which promotes Sustainable Development Goal (SDG) 14 – “Conserve and Sustainably use the oceans, seas and marine resources for sustainable development” (United Nations Development Programme, 2015; FAO, 2020). The aquaculture industry in Africa requires least-cost formulated feeds to sustain constant growth, as aquaculture in Africa has been identified as one of several initiatives that can be used to address and alleviate poverty and food insecurity (Hishamunda & Ridler, 2006a; Firth, 2018). Bearing this in mind, there is a need for more focussed research on diet supplements that can assist with sustaining growth. One such a feed supplement candidate is a phytocannabinoid known as Cannabidiol (CBD).

During the last couple of years there has been an increased focus on research on CBD, since its isolation in 1940 by Adams *et al.* (1940) from marijuana, and by Jacob & Todd (1940) from Indian Hemp resin. Since then, there has been considerable advances in studying CBD and its effects on anxiety and mental disorders (Michoulam *et al.*,1963). Zuardi (2008) reported that between 1973 and 1982, there has been a decline in studies focusing on CBD, resulting in about 11 publications per year since 1968. However, between 2003 and 2007, 225 publications on CBD were produced, a dramatic increase from about 55 in the 1998-2002 period. Only one study by Saoud *et al.* (2018), focused on industrial hemp and cannabis as potential feed supplements in Nile tilapia (*Oreochromis niloticus*). A limitation of this study, however, was that it did not focus on an isolated phytocannabinoid. Hemp is known to contain a variety of phytocannabinoids, with cannabidiol (CBD) being predominantly produced (Pisanti *et al.*, 2017). According to de Meijer & Hammond (2005), inbred offspring crossed with true breeding THC or CBD plants resulted in the European fibre hemp populations producing predominantly cannabigerol (CBG) instead of CBD. Hence, one has to be sure which plant variety are used.

Therefore, in this study the aim was to use CBD isolate from as opposed to using the whole plant. The CBD that were used in the present study was isolated from the rest of the available cannabinoids to get the purest form of CBD, also known as CBD isolate, and not directly as a result of cold-pressed seeds as in previous studies. The effects observed in the present study is as a result of CBD isolate and no other phytocannabinoids because CBD isolate were mixed with hemp seed oil, containing no other phytocannabinoids, as the basis of the CBD oil product.

There is very little research done on phytocannabinoids and their effects on fish to compare the treatment effects in the present study.

The aims of this study were therefore to assess the effect of CBD on the knowledge on the effects of CBD on the performance of *O. niloticus* by collecting data on feed intake, weight gain, specific growth rate, and feed conversion ratio. Furthermore, the study evaluated the effect of CBD on haematological parameters such as white blood cells, red blood cells, and platelets.

References

Adams, B. R., Hunt, M., & Clark, J. H. 1940. Cannabidiol. J. Am. Chem. Soc. 62.

Béné, C., Barange, M., Subasinghe, R., Pinstrup-Andersen, P., Merino, G., Hemre, G. I., & Williams, M. 2015. Feeding 9 billion by 2050 – Putting fish back on the menu. Food Secur. 7, 261–274 https://doi.org/10.1007/s12571-015-0427-z.

El-Sayed, A. F. M. 2006. Tilapia culture in salt water: environmental requirements, nutritional implications, and economic potentials. Av. en Nutr. Acuícola VIII, 95–106.

Firth, D. C. 2018. Temporal and inter-species variations in the proximate and contaminant compositions of farmed mussels , Choromytilus meridionalis and Mytilus galloprovincialis , from Saldanha Bay , South Africa. , 1–123.

Food and Agriculture Organization of the United Nations. 2020. The State of World fisheries and aquaculture in review. FAO.org, Rome.

High Level Panel of Experts (HLPE) on Food Security and Nutrition. 2014. Sustainable fisheries and aquaculture for food security and nutrition. Rome.

Hishamunda, N., & Ridler, N. B. 2006. Farming fish for profits: A small step towards food security in sub-Saharan Africa. Food Policy 31, 401–414 https://doi.org/10.1016/j.foodpol.2005.12.004.

Jacob, A., & Todd, A. R. 1940. No Title. J. Chem. Soc.

Madage, S. S. K., Medis, W. U. D., & Sultanbawa, Y. 2015. Fish Silage as Replacement of Fishmeal in Red Tilapia Feeds. J. Appl. Aquac. 27, 95–106 https://doi.org/10.1080/10454438.2015.1005483.

De Meijer, E. P. M., & Hammond, K. M. 2005. The inheritance of chemical phenotype in Cannabis sativa L. (II): Cannabigerol predominant plants. Euphytica 145, 189–198 https://doi.org/10.1007/s10681-005-1164-8.

Michoulam, R., Shvo, Y., & Hashish, I. 1963. The structure of cannabidiol. Tetrahedron 19, 2073–2078.

Pisanti, S., Malfitano, A. M., Ciaglia, E., Lamberti, A., Ranieri, R., Cuomo, G., Abate, M., Faggiana, G., Chiara Proto, M., Fiore, D., Laezza, C., & Bifulco, M. 2017. Cannabidiol: State of the art and new challenges for therapeutic applications. https://doi.org/10.1016/j.pharmthera.2017.02.041.

Saoud, I., Babikian, J., Nasser, N., & Monzer, S. 2018. Effect of cannabis oil on growth performance, haematology and metabolism of Nile Tilapia Oreochromis niloticus. Aquac. Res. 49, 809–815 https://doi.org/10.1111/are.13512.

Tacon, A. G. J., & Metian, M. 2015. Feed Matters: Satisfying the Feed Demand of Aquaculture. Rev. Fish. Sci. \& Aquac. 23, 1–10 https://doi.org/10.1080/23308249.2014.987209.

United Nations Development Programme. 2015. Sustainable Development Goals., 17.

Zuardi, A. W. 2008. Cannabidiol : from an inactive cannabinoid to a drug with wide spectrum of action Canabidiol : de um canabinóide inativo a uma droga com amplo espectro de ação. Rev. Bras. Psiquiatr. 30, 271–280 https://doi.org/10.1590/S1516-44462008000300015.

Chapter 2 Literature Review

2.1. Overview of international aquaculture production

Over the last 50 years, scientific investigations have contributed to an improved understanding of the functioning of aquatic ecosystems to ensure sustainable management of these systems (FAO, 2020). The significance of utilising fisheries and aquaculture responsibly were recognised 25 years after it has been published in the Code of Conduct (CC) for Responsible Fisheries (FAO, 2020). FAO (2020) stated that the CC provided guidance on the development of international instruments, policies and programmes to support responsible management efforts globally, regionally and nationally. In particular, Sustainable Development Goal (SDG) 14 – "Conserve and sustainably use the oceans, seas and marine resources for sustainable development" along with relevant SDG's (United Nations Development Programme, 2015b), were addressed and prioritised since 2015 (Transforming our world: the 2030 Agenda for Sustainable Development, 2015) with the abovementioned policies, programmes and international instruments which was guided by the abovementioned CC.

As a result of the re-prioritisation of SDG 14 and other related SDG's, science-based fisheries and aquaculture management policies are now being widely implemented and accepted as minimum criteria for sustainable fisheries and aquaculture (FAO, 2020). According to the FAO (2020), this cannot be achieved without generically applicable production protocols for international fish trade and usage.

2.2. Aquaculture Production

In 2018, global fish production (fisheries and aquaculture) reached 179 million tonnes, as indicated in Table 2.1 and Figure 2.1 (FAO, 2020). According to the FAO (2020) the first sale of the global fish production was valued at USD 401 billion. Of this first sale, aquaculture production contributed 82 million tonnes (46% of global fish production), valued at USD 250 billion (FAO, 2020). The global fish production used for human consumption were 156 million tonnes of which 52% came from aquaculture production. The remaining 23 million tonnes of the global fish production, for the same year, were for non-food uses, mainly for fishmeal production (Figure 2.2) (FAO, 2020). Apart from the 82 million tonnes produced by aquaculture activities in 2018, the FAO (2020) reports that "32.4 million tonnes of aquatic algae and 26 000

tonnes of ornamental seashells and pearls" were produced as well, which brings the total aquaculture production to about 114.5 million tonnes. This is an all-time high when compared to previous years.

Table 2.1 World fisheries and aquaculture production, utilisation, and trade for 1986-2018. (FAO, 2020).

	1986–1995	1996–2005	2006–2015	2016	2017	2018
	Average per year					
	(million tonnes, live weight)					
Production						
Capture						
Inland	6.4	8.3	10.6	11.4	11.9	12.0
Marine	80.5	83.0	79.3	78.3	81.2	84.4
Total capture	**86.9**	**91.4**	**89.8**	**89.6**	**93.1**	**96.4**
Aquaculture						
Inland	8.6	19.8	36.8	48.0	49.6	51.3
Marine	6.3	14.4	22.8	28.5	30.0	30.8
Total aquaculture	**14.9**	**34.2**	**59.7**	**76.5**	**79.5**	**82.1**
Total world fisheries and aquaculture	**101.8**	**125.6**	**149.5**	**166.1**	**172.7**	**178.5**
Utilization[2]						
Human consumption	71.8	98.5	129.2	148.2	152.9	156.4
Non-food uses	29.9	27.1	20.3	17.9	19.7	22.2
Population (*billions*)[3]	5.4	6.2	7.0	7.5	7.5	7.6
Per capita apparent consumption (*kg*)	13.4	15.9	18.4	19.9	20.3	20.5
Trade						
Fish exports – in quantity	34.9	46.7	56.7	59.5	64.9	67.1
Share of exports in total production	*34.3%*	*37.2%*	*37.9%*	*35.8%*	*37.6%*	*37.6%*
Fish exports – in value (*USD billions*)	37.0	59.6	117.1	142.6	156.0	164.1

[1] Excludes aquatic mammals, crocodiles, alligators and caimans, seaweeds and other aquatic plants. Totals may not match due to rounding.

[2] Utilization data for 2014–2018 are provisional estimates.

[3] Source of population figures: UN DESA, 2019.

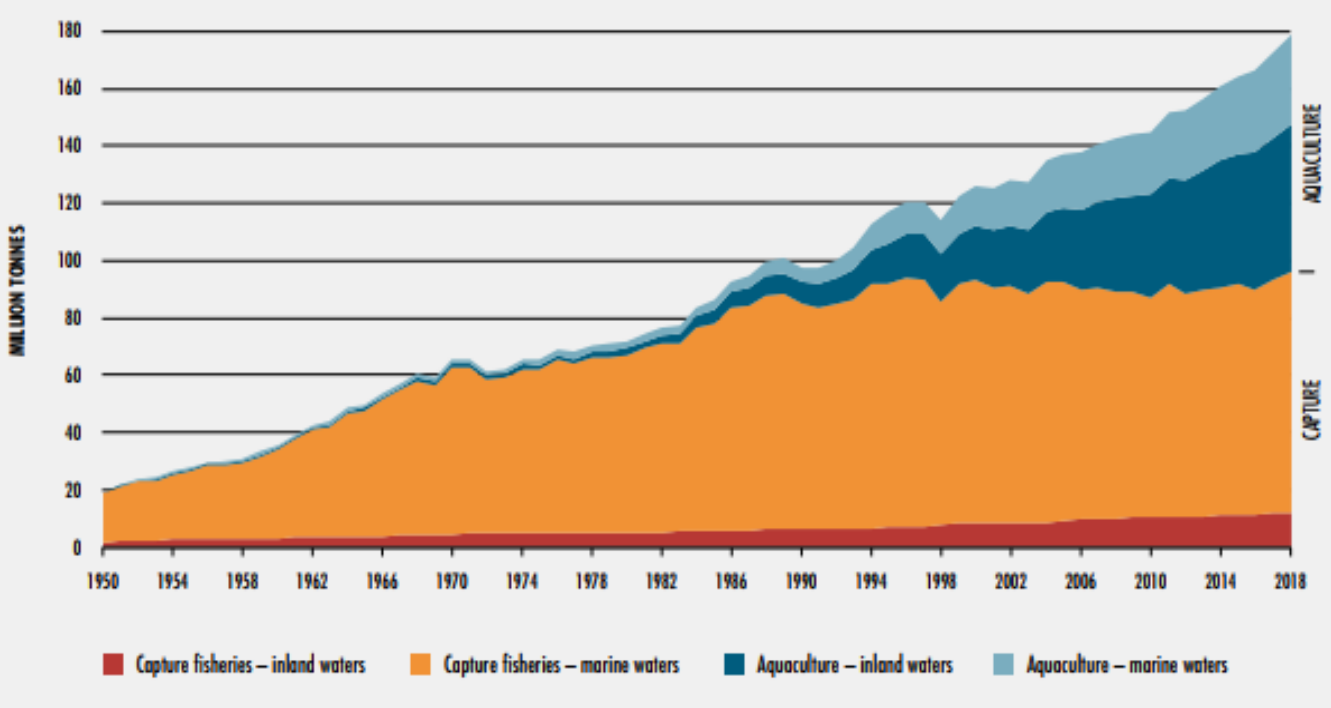


Figure 2.1 World capture fisheries and aquaculture production from 1950-2018 (FAO, 2020).

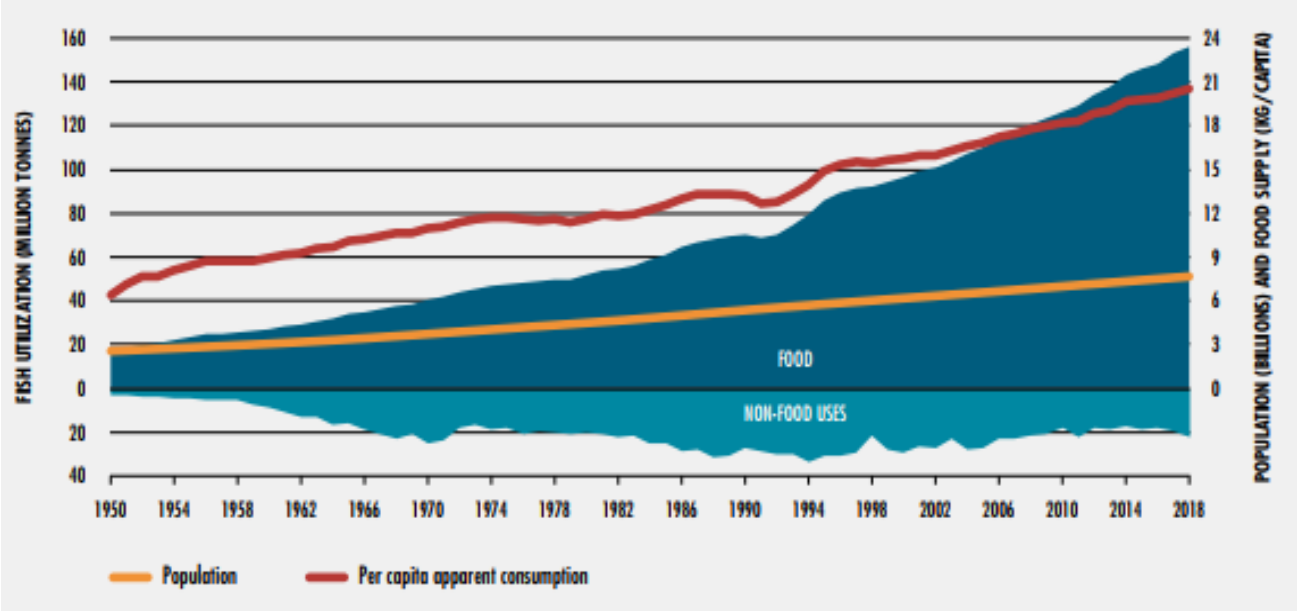


Figure 2.2 World fish utilization and apparent consumption from 1950-2018 (FAO, 2020).

Aquaculture production in 2018 was dominated by finfish production, with about 54.3 million tonnes – 47 million tonnes and 7.3 million tonnes from inland aquaculture and marine and coastal aquaculture, respectively. Molluscs which consisted mainly of bivalves contributed 17.7 million tonnes, while crustaceans contributed only 9.4 million tonnes (FAO, 2020). In general, the FAO (2020) reported a slight decrease in finfish production of 97.2 % in 2000 to 91.5 % in 2018. However, production of other species groups like crustaceans which includes shrimps, crayfish and crabs increased between the same period reported above (FAO, 2020).

In Africa, Egypt is reported to be the top producer among other African countries with more than 750 000 tonnes of fish production per annum (James, 2019). In many African countries, cage culture of Tilapia forms the bulk of fish production resulting in thousands of tonnes of Tilapia being produced (James, 2019). In Nigeria, catfish contributes the highest in terms of production, it is impossible to quantify the statistics as the majority of contributions comes from backyard or small commercial ventures (James 2019). Figure 2.3 represents one of the numerous small- to medium-scale commercial enterprises in Zambia and Uganda.

Figure 2.3 Medium-scale commercial farm in Zambia (James, 2019).

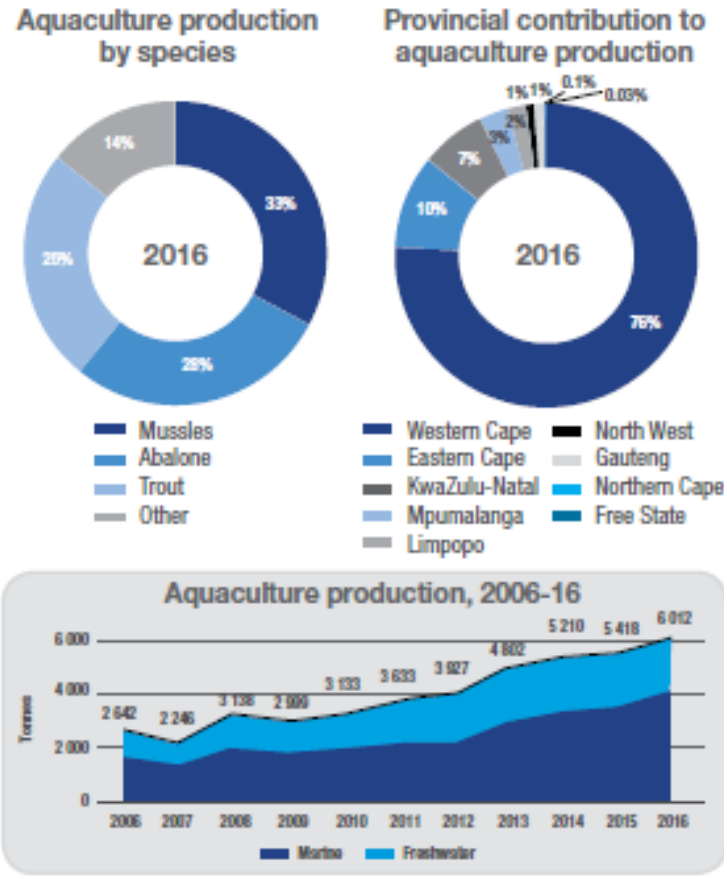
2.3. South African Aquaculture

The aquaculture sector in South Africa is comparatively small, when compared to the global aquaculture industry (South African Department: Trade and Industry, 2020). According to The

South African Department of Trade and Industry (2020), the South African aquaculture sector contributes about 0.8% to the country's fish production, which accounts for less than 0.2% of the country's GDP. The government earmarked the aquaculture sector as a priority sector as a result of its growth potential (South African Department: Trade and Industry, 2020).

South Africa is reported to have about 200 marine and freshwater farms (South African Department: Trade and Industry, 2020). According to the Department of Trade and Industry (2020), aquaculture in South Africa more than doubled between 2006 and 2016. The major farmed species are abalone (*Haliotis midae*, 1758), catfish (*Clarius gariepinus)*, spotted grunter (*Pomadasys commersonnii*), dusky kob (*Argyrosomus japonicus*), saltwater Tilapia (*Oreochromis mossambicus*), ocean trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), barramundi (*Lates calcarifer*)), rainbow trout (*Oncorhynchus mykiss*), Mediterranean mussels (*Mytilus galloprovincialis*) and Black mussels (*Choromytilus meridionalis*), Pacific oysters (*Crassostrea gigas)* and Tilapia (*Oreochromis mossambicus*, *Oreochromis niloticus,* and *Oreochromis aureus).*

According to the Department of Trade and Industry (2020), South Africa exported fish- and seafood-related products worth more than R6.6 billion. About a third of these exports consist of molluscs of which abalone is the biggest output (South African Department: Trade and Industry, 2020). According to DAFF (2015) and Firth (2018), in 2011 abalone mariculture was the most valuable subsector with a total production of about 1 036 tonnes followed by mussels and oysters at 570 and 69 tonnes, respectively. Mussel production surpassed abalone production in 2014 when the total marine aquaculture output increased significantly from 1 883 to 3 417 (The Department of Agriculture Forestry and Fisheries, 2015, 2016; Firth, 2018). Although it seems in Figure 2.4 that mussels dominate the aquaculture sector in South Africa, DAFF (2015, 2016) and Firth (2018) reported that abalone still remains the most valuable sub-sector.



2.4. Nile tilapia (*Oreochromis niloticus*)

2.4.1. Species Background

Nile Tilapia (*O. niloticus*) is a benthopelagic fish, which means that it lives and feeds near the bottom, midwaters and sometimes near the surface. *Oreochromis niloticus* can be easily distinguishable from other tilapias via the colour patterns on their bodies and fins (DAFF, 2018). Nile Tilapia were cultured since Egyptian times in earthen ponds (FAO, 2016). Distribution of Nile Tilapia occurs naturally in Africa and coastal rivers of Israel (DAFF, 2018).

Furthermore, uncontrolled breeding in the abovementioned ornamental ponds led to “excessive recruitment, stunting and a low percentage of marketable-sized” Tilapias (FAO, 2021). Only in the 1970’s hormonal sex-reversal techniques were developed which allowed mono-sex male Tilapia to be raised to “uniform, marketable sizes” (FAO, 2021). According to DAFF (DAFF, 2018a), Nile Tilapia is the most widely farmed tilapia fish representing about 83% of all Tilapia production and is marketed both fresh and frozen. However, in South Africa, Nile Tilapia is an invasive species

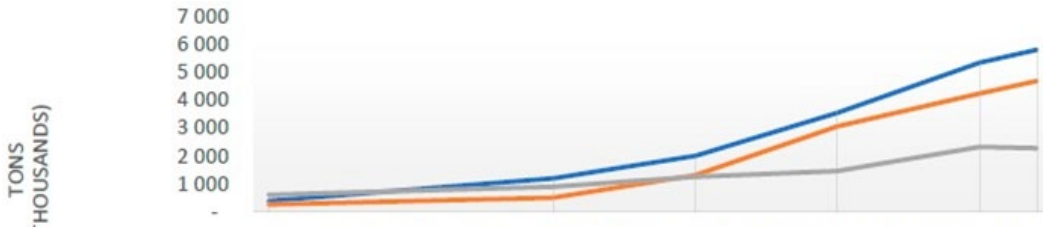
that threaten the existence of the indigenous Tilapia species known as Mozambique Tilapia as a result of several attributes that allows it to outcompete Mozambique Tilapia (DAFF, 2018).

According to the feasibility study for Nile and Mozambique Tilapia by DAFF (2018), attributes that make Nile Tilapia an attractive culture species include; high tolerance of poor water quality parameters and high stocking density, increased performance on low protein formulated feeds, acceptance of high plant protein feeds, increased disease resistance, better consumer appeal than other freshwater fish. Moreover, it can be cultured in a wide range of culture systems, and it is considered the only economically viable Tilapia species (Kentucky State University Aquaculture, 2015).

In South Africa, unlike many other countries the climatic and geographic distribution allows for massive temperature differences (DAFF, 2018). Temperature is a key factor that impacts the feasibility of any aquaculture venture, whether it is to heat or cool water for production. Water temperature influences the type of production system that can be used, low and high water temperatures can be easily maintained in certain systems to maintain the water temperature (DAFF, 2018). Nile Tilapia in particular has a lower and upper lethal temperature limit of 11˚C and 42˚C, respectively (FAO, 2021). According to the FAO (2016), the preferred temperature range for optimum growth are between 31 to 36˚C. This temperature range as well as the temperature limits for Nile Tilapia makes it very difficult if not near impossible to farm in open outdoor ponds, as South African temperatures are not suitable to allow for optimum growth of Nile Tilapia (James, 2012).

2.4.2. Nile Tilapia Production

According to Hishamunda *et al.* (2006b) half of the world's food fish are produced by means of aquaculture. Out of the most commonly farmed fish, Tilapia (Nile and Mozambique) is the second most farmed fish with Carp being the most commonly farmed fish globally (DAFF, 2018). In comparison with key species like Atlantic salmon and Catfish, Tilapia has been dominating since 2000, as can be seen in Figure 2.1.



	1990	2000	2005	2010	2015	2017
Tilapia	379 000	1 190 000	1 992 000	3 541 000	5 339 000	5 798 000
Catfish	250 000	500 000	1 302 521	3 056 400	4 228 000	4 676 000
Atlantic Salmon	600 000	893 704	1 250 700	1 455 200	2 314 600	2 269 500

Figure 2.5 Global production of Tilapia, Catfish and Atlantic Salmon – comparative analysis for 1990-2017 (Tveteras, 2016; DAFF, 2018).

From Figure 2.5 one can see that a production level of just under 1.2 million tons grew to just under 5.8 million tons in 2017, an increase of about 483%. This equates to an average yearly increase of about 28%. According to DAFF (2018), this increase can be attributed to the technological improvements and market growth in the aquaculture industry for Tilapia.

According to DAFF (2018), in terms of Tilapia production, the dominating continents during 2015 were:

- Asia contributed about 78% to global Tilapia production which equates to about 4 million tons
- Africa contributed about 14% to global Tilapia production, equating to more than 700 thousand tons, and
- South America contributed about 9% to global Tilapia production which equates to about 450 thousand tons (DAFF, 2018).

The most recent aquaculture production data that are available for South Africa is that of 2015, almost 6 years of production data are missing. This makes it a bit difficult to comment on the aquaculture sector in South Africa. However, based on the data available for 2015 (Table *2.2*), the total number of farms that operated in South Africa in 2015 was only 74. The report by DAFF (2016) does not provide detailed information on what type of Tilapia farm it is, hatchery or grow-out. As of 2021, with the worst part of the Covid-19 pandemic passed, one can expect that this number might have grown to be more than 74 since 2015 but declined because of the impacts the pandemic had on the aquaculture sector. According to Dempsey (2021), as of January 2021 there were less than 100 Tilapia farmers in South Africa which are mostly consisting of “small pond farmers” and a few “small- to medium-scale commercial farms”.

Table 2.2 Freshwater aquaculture farms by sub-sector in provinces in South Africa that operated in 2015 DAFF (2016).

Species	EC	FS	GP	KZN	LP	MP	NC	NW	WC	Total
Tilapia	2	0	20	5	16	14	1	14	2	74
Trout	2	0	0	5	0	18	0	0	13	38
Catfish	1	7	1	0	3	0	0	1	0	13
Marron Crayfish	1	0	0	0	0	0	0	0	0	1
Carp	0	0	1	0	0	0	1	0	1	3
Koi carp	0	2	5	2	0	1	0	0	1	11
Ornamental species	0	1	4	3	1	0	0	0	3	12
Total	6	10	31	15	20	33	2	15	20	152

The data available for the period 2006-2015 (Table 2.3), one can see that up until 2008 there were no Tilapia production in South Africa at all. Only from 2009 – 2015 production increased from 10 tons per year to 325.29 tons per year, respectively. At this rate, the contribution of South African freshwater Tilapia aquaculture to the African or Global freshwater Tilapia aquaculture industry can be considered negligible.

Table 2.3 Freshwater aquaculture production trend per sub-sector in South Africa in 2015 DAFF (2016).

Sub-sector	Year and production(tons)										Total production (tons)
	2006	2007	2008	2009	2010	2011	2012	2013	2014	**2015	*****
Tilapia	0	0	0	10	10	100	234.17	289.71	289.71	325.29	1258.88
Trout	807	658	943	948.62	950	1199	1428	1497.3	1497.3	1497	11425.22
Catfish	180	180	180	180	180	160	0	0	0	0	1060
Marron crayfish	0.2	0.4	0.4	0.4	0.8	0.8	3.5	5	5	4	20.5
Totals	987.2	838.4	1123.4	1139.02	1140.8	1459.8	1665.67	1816.41	1792.01	1826.29	13764.6

2.4.2. Factors affecting growth performance in nile tilapia

Just like in many animal production systems in agriculture, the species that has attributes that makes it more favourable to farm with over other existing species, is most often the most desired for farmers and/or producers. This is the case with Nile Tilapia. According to DAFF (2018), Nile Tilapia's "rapid growth, late age of sexual maturity and planktivorous feeding habits" makes it more attractive as a culture species.

Temperature is probably the most important factor that impacts growth and reproduction, especially in Nile Tilapia. On its own, temperature influences growth, reproduction and sometimes can even influence the integrity of the production system if the water temperature is not in line with the optimal water temperature range suggested by literature for the species one intends to culture. According to DAFF (2018), the optimal water temperature ranges for Nile Tilapia culture is 28 – 36˚C. Besides the direct impacts of temperature, there are also indirect impacts brought on by temperature that influences the well-being, growth, and reproduction of Nile Tilapia.

One of these indirect impacts are on pH levels of culture water. According to DAFF (2018), Nile Tilapia can survive in water with a pH level range between 5 to 10, but optimally in a range of 6 to 9. According to Campbell (1985) and Boundless (2015), Le Chatelier's principle can be an easy explanation why and how pH levels of culture water gets influenced when water temperatures change. Simply put, the pH level of water decreases with an increase in temperature and vice versa (Westlab, 2017).

During the nitrification process oxygen gets consumed as the ammonia gets oxidised and if the DO level is low, the nitrification process competes against respiration in which it gets the lowest oxygen leading to low oxygen levels and high ammonia levels in the process (United States Environmental Protection Agency (EPA), 2019). According to DAFF (2018), the oxygen requirements for Nile Tilapia in culture systems should be maintained above 1 mg/l although it can survive in DO levels below 1 mg/l.

The increased ammonia will lead to increased pH levels in the production system and so an increased pH will lead to the increase of un-ionised ammonia (Wurts, 1992). Nile Tilapia may be considered a hardy fish, but it will not survive any unionised ammonia concentration below 2 mg/L (DAFF, 2018). Unionised ammonia as low as 0.08 mg/l starts to reduce feed consumption which inevitably leads to mortalities at prolonged exposure (DAFF, 2018). According to El-Sayed (2006), the recommendation is that ammonia levels should be maintained below 0.1 mg/l.

From the above, one can deduce that a change in water temperature outside the range of optimal water temperature for Nile Tilapia can influence the DO level within the water, the pH of the water and the DO of the water can influence the unionised ammonia concentration. It is therefore vital that these water quality parameters should be kept within its optimal ranges for Nile Tilapia culture.

2.4.3. Body condition of nile tilapia

The body condition factor and the weight-length relationship, represented by Equations 2.1 and 2.2, respectively, of fish is an indicator of its health, wellbeing and nutritional status (Froese, 2006; Joscelyne, 2020). According to Ricker (1975), Nash *et al.* (2006) and Omogoriola *et al.* (2011), a higher condition factor is desired as this is seen as an indicator of good health, well-being and nutrition, i.e. the higher the condition factor, the healthier the fish. The a (intercept) and b (slope) values in Equations 2.1 and 2.2 were derived from the regression equation represented by Equation 2.3.

The b-value suggests what type of growth, isometric or allometric, were experienced by fish. A b = 3 is an indication of isometric growth and any deviation from that is allometric growth (Ricker, 1975). Isometric growth is described by Ricker (1975) and Omogoriola *et al.* (2011) as a uniform increase in body weight with an increase in unit of length. The isometric b-value according to Jones *et al.* (1999) represents an assumption that was made by Fulton's condition factor. Froese (2006) argued that the assumption by Fulton's condition factor, is not correct at all times as b-values not equivalent to three ($b \neq 3$) results in allometric growth. A b-value greater or smaller than 3 are known as positive or negative allometric growth, respectively (Omogoriola *et al.*, 2011). Positive allometric growth is an indication that the fish grows faster in weight than in length and

these fish normally exhibit a body shape that's more round (Joscelyne, 2020). Negative allometric growth is the exact opposite of positive allometric growth, where the fish grows faster in length than in weight, making the fish appear to be more slender (Joscelyne, 2020). The b-value is not an absolute constant value that's affected only by a singular factor, it is known that factors like age, sex, season, maturation, fullness of the gut, feed, fat reserves and musculature can also play a role in the type of growth experienced by fish (Le Cren, 1951; Barnham & Baxter, 1998; Khallaf *et al.*, 2003; Luckhoff, 2005; Hossain *et al.*, 2006; Tarkan *et al.*, 2006; Froese, 2006; Muchlisin *et al.*, 2010; Joscelyne, 2020).

$$K = \frac{100W}{L^b} \quad \textbf{2.1}$$

Where K = Condition Factor (Pauly, 1984; Gomiero & De Souza Braga, 2005)

W = Final Body Weight (FBW) in gram (g)

L = Total Length of fish in centimetres (cm)

b = exponent of the length-weight equation

$$W = aL^b \text{ (Pauly, 1984)} \quad \textbf{2.2}$$

Where W = Weight of fish (g)

a = exponent describing the rate of change of weight with length (intercept)

L = Total Length of fish in centimetres (cm)

b = Weight at unit length (slope)

$$log\, W = b\, log\, L + log\, a \text{ (Zar, 1984)} \quad \textbf{2.3}$$

Where W = Weight of fish (g)

b = Weight at unit length (slope)

L = Total Length of fish in centimetres (cm)

a = exponent describing the rate of change of weight with length (intercept)

2.5. Endocannabinoid System (ECS)

The endocannabinoid system (ECS) is complex and widely distributed throughout the body. According to Hazzah *et al.* (2020) the ECS is a regulatory system that provides essential mechanisms that ensures feedback and maintains biological balance throughout the body. The ECS has been identified in a number of species; humans, birds, canines (McPartland *et al.*, 2006b), and fish (McPartland *et al.*, 2006a; Silver, 2019).

The functions of the ECS has been described as “relax, eat, sleep, forget, and protect” for many years (Di Marzo, 1998; Hazzah *et al.*, 2020). However, as research progressed into the ECS, its essential role in modulatory functions like “appetite, digestion, energy balance, sleep patterns, immune status, inflammation and emotional responses” in complex and diverse systems like the brain, endocrine and immune tissues, has been noticed (Di Marzo, 1998; Komorowski & Stepień, 2007; Hazzah *et al.*, 2020).

A typical ECS can be visualised as a system consisting of three components: endogenous ligands of cannabinoid receptors (CBRs) also known as endocannabinoids (eCBs), CBRs, and enzymes responsible for the transport, activation and breakdown of eCBs (Hazzah *et al.*, 2020). Figure 2.7 represents the typical actions of the eCB Anandamide (AN) and 2-arachidonylglycerol (2-AG) on the CB1 and CB2 receptors that exist in the presynaptic neuron of the central and peripheral nervous system (VanDolah *et al.*, 2019). The compounds that are shaded in green (Figure 2.7) are phytocannabinoids that affect the function of the ECS under normal circumstances. BCP = β-caryophyllene; GABA = γ-aminobutyric acid; TRPV = transient receptor potential vanilloid (VanDolah *et al.*, 2019).

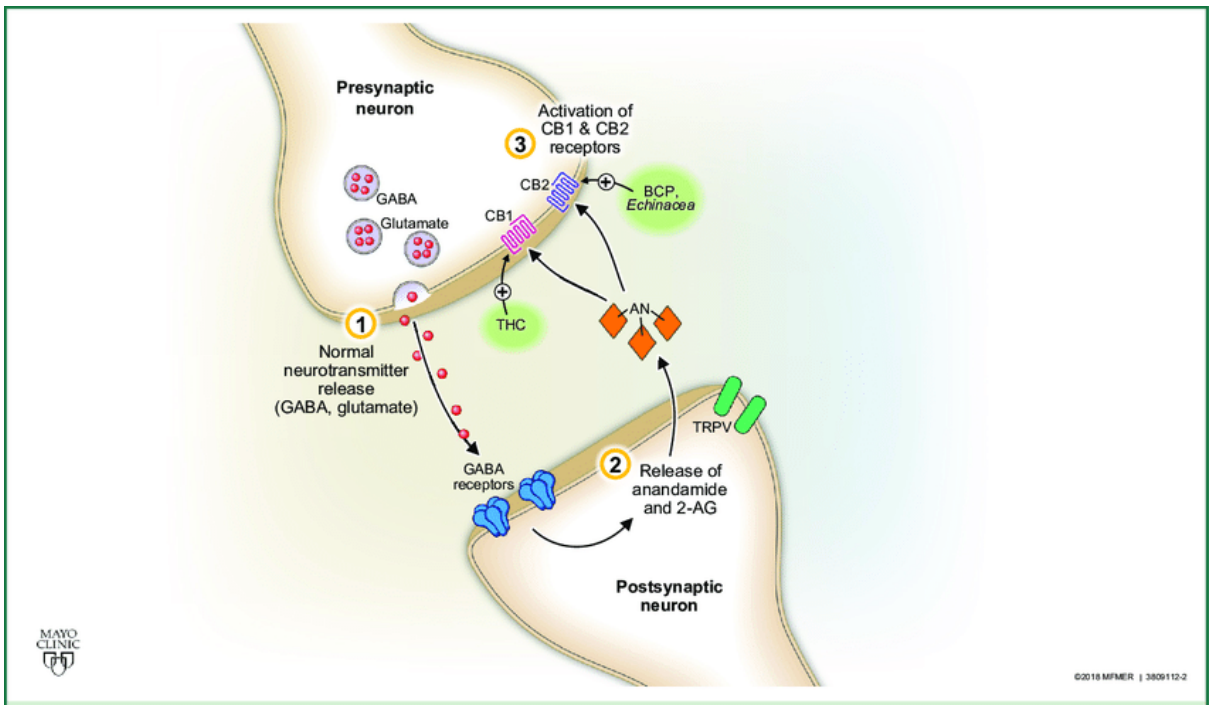


Figure 2.6 A modulation of the ECS by phytocannabinoids (Pertwee, 2008; Pertwee et al., 2010; Zou & Kumar, 2018; Van Dolah et al., 2019).

Subsequently, potential effects of CBD on feeding behaviour of rats and the effects of chronic administration of CBD on body weight were studied, that found that rats lost weight over the duration of the study (Wiley et al., 2005; Riedel et al., 2009; Izzo et al., 2009; Ignatowska-Jankowska et al., 2011). Saoud et al. (2018) investigated the effects of industrial hemp as a treatment on feeding behaviour of Nile Tilapia and found no significant effects that indicates that industrial hemp can increase appetite. Studies by Wiley et al. (2005), Riedel et al. (2009), Izzo et al. (2009), Ignatowska-Jankowska et al. (2011) and Saoud et al. (2018) showed that administering CBD to mice, rats, and fish, respectively, cause weight-loss.

The feeling of fullness after a meal or better known as satiety are modulated through the hypothalamic pro-opiomelanocortin (POMC) neurons (Silver, 2019). When the CB1 receptor gets activated by the AN ligands or by THC (Figure 2.7), the POMC neurons gets inhibited (Silver, 2019). Without the POMC signals to indicate satiety, it causes an increase in appetite which renders the hyperphagia experienced or noticed when cannabis with high THC content are used (Silver, 2019). According to Silver (2019), POMC neurons gets inhibited as a result of a blockade of the α-melanocyte-stimulating hormone (α-MSH), an appetite suppressant signal molecule, which literally stops the signalling to the brain to indicate satiety. Furthermore, Orexin-A cause an increase in the levels of 2-AG (Figure 2.7) which induce hyperphagia (Morello *et al.*, 2016; Silver, 2019). As α-MSH decrease, 2-AG increases. Silver (2019) notes that human cannabis users experiencing hyperphagia, generally eat more calories as a result of the high THC content of the cannabis. However, inexplicably, based on published studies, cannabis users are generally slimmer than non-users (Silver, 2019). This can be explained by fasting insulin and insulin sensitivity being increased in chronic cannabis users, contrary to non-users (Le Foll *et al.*, 2013;

Silver, 2019). The results obtained by the abovementioned studies on CBD, on different animal species, might render the same results, but it still contributes to the limited available research on the ECS. The assumption that all animal species possess an ECS that is alike cannot be affirmed as Silver (2019) highlights the lack of cannabinoid binding in the sea anemone (*A. albocinta*) and the sponge (*T. aurantium*). According to Silver (2019) only CB1 receptors were detected on CBD and not CB2 receptors. This warrants studies that investigates the effects of cannabinoids on the growth, health and well-being of fish and other animal species on which published research is lacking.

2.6. *Cannabis sativa* and Cannabidiol (CBD)

2.6.1. Cannabis sativa L. Short Overview

Cannabis is a genus of herbaceous flowering plants in the Cannabaceae family. *Cannabis sativa (Linnaeus, 1753)* also known as hemp, marijuana, pot, hashish and weed (Zuardi, 2008). Cannabis is said to have been cultivated for more than 5000 years for uses such as paper, fibre, medicine and even clothing (Hazzah *et al.*, 2020).

According to Russo *et al.* (2007), the beneficial uses for the plants as a result of the secondary products of the plant is for “chronic pain, spasticity, seizure disorders, and cancers”. However, its effects on these ailments are ill understood and not sufficient research has been done (Russo, 2007; Small, 2017; Saoud *et al.*, 2018). To put this into perspective, the human cannabis market alone, excluding its veterinary uses, are projected to grow from USD 12.581 Billion in 2018 to USD 36.903 Billion by the year 2024 (Wood, 2019). Which should warrant more research into the effects of *Cannabis sativa* and its secondary products.

2.6.2. Phytocannabinoids

Cannabis sativa is characterized by more than 750 bioactive compounds (Upton *et al.*, 2014), with more than 150 of these compounds consisting of phytocannabinoids (Shahbazi *et al.*, 2020). Phytocannabinoids can be found in resin within flowers, leaves and seeds (Atakan, 2012; Hazzah *et al.*, 2020; De Briyne *et al.*, 2021).

Before cannabinoids can be utilised for any of its various uses, it is in the form of carboxylic acids (Hazzah *et al.*, 2020). Tetrahydrocannabinol (THC) and Cannabidiol (CBD) in their carboxylic acid forms, Figure 2.8 and Figure 2.9, respectively, have to be converted to their stable and neutral forms known as THC and CBD by influence of heat and light in order to remove the carboxyl

groups and releasing CO_2 in the process, more commonly known as decarboxylation (Wang *et al.*, 2016b; Hazzah *et al.*, 2020).

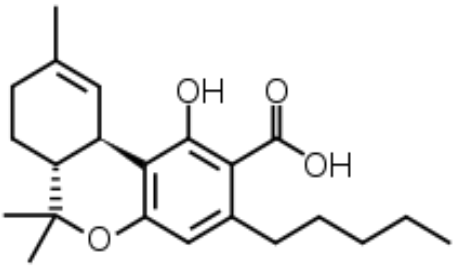


Figure 2.7 Skeletal chemical structure of tetrahydrocannabinolic acid (THCA) (Moreno-Sanz, 2016).

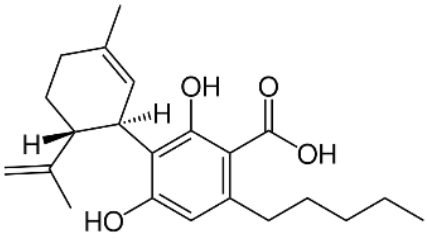


Figure 2.8 Skeletal chemical structure of cannabidiolic acid (CBDA) (Senegal, 2020).

The two major phytocannabinoids, THC and CBD in Figure 2.10 and Figure 2.11, respectively, are the most researched and known phytocannabinoids (Hazzah *et al.*, 2020). THC and CBD are chemical isomers and both bind to receptors in the brain that affect sleep, mood and anxiety, but THC alone has psychoactive elements making their physiological effects differ significantly (Hazzah *et al.*, 2020).

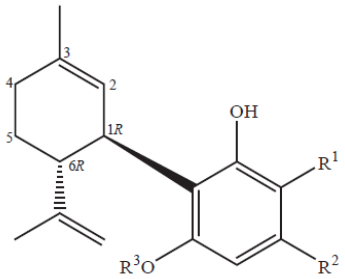


Figure 2.9 Chemical structure of Cannabidiol (CBD) (Moreno-Sanz, 2016).

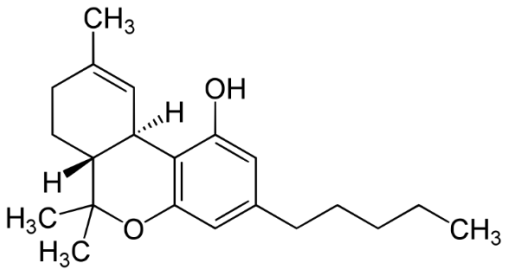


Figure 2.10 Chemical structure of Tetrahydrocannabinol (Moreno-Sanz, 2016).

2.6.2.1. Tetrahydrocannabinol (THC)

Tetrahydrocannabinol (THC) binds to the CB1 and CB2 receptors as an agonist and can have therapeutic affects like muscle relaxation, analgesia as well as anticonvulsant properties (Shahbazi *et al.*, 2020). The binding to the CB2 receptor allows it to cause immunomodulatory and anti-inflammatory properties (Hazzah *et al.*, 2020). The anti-inflammatory properties by THC are reported to have a greater effect than aspirin and hydrocortisone (Evans, 1991; Hazzah *et al.*, 2020). Additional medical benefits of THC reported are; “bronchodilation, gastrointestinal support (including inflammatory bowel disease), reduced intraocular pressure, antineoplastic effect, neuroprotective activity, and sleep support” (Williams *et al.*, 1976; Hampson *et al.*, 2000; Tomida *et al.*, 2006; Naftali *et al.*, 2013; Bowles *et al.*, 2017; Couch *et al.*, 2018; Hinz & Ramer, 2019; Hazzah *et al.*, 2020).

2.6.2.2. Cannabidiol (CBD)

CBD, contrasting to THC, does not cause any intoxicating effects. However, the lack of intoxicating effects does not mean that it is not psychoactive. Hazzah *et al.* (2020) reports that the CBD molecule is able to cause changes in brain function that is clinically detectable changes in ”perception, mood, and consciousness” and are frequently used to cause anxiolytic and antidepressant effects, that can be seen as psychoactive. Many studies, cited in McPartland *et al.* (2015), noticed the antagonistic and modulatory interaction of CBD on THC leading to the assumption that CBD applies a pharmacodynamic barrier on THC, reducing and sometimes even inhibiting secondary effects like “psychotoxicity, paranoia, and tachycardia” (Hazzah *et al.*, 2020). Elmes *et al.* (2015), Chung *et al.* (2019), and Hazzah *et al.* (2020) is of the opinion that these interactions by CBD molecules occur across a number of mechanisms during the competition for fatty acid binding proteins (FABPs) and ”negative allosteric modulation of eCB receptors, competitive inhibition of the cytochrome P450 enzyme complex”. Elmes *et al.* (2015) found that when CBD and THC are co-administered orally, CBD hinders the function of cytochrome P450, slowing down the metabolism of THC to 11-hydroxy-Δ^9-THC. According to McPartland *et al.*

(2015) a descriptive study conducted on rats by Browne & Weissman (1981), the latter metabolite is four times more psychoactive than THC.

CBD are considered to be an antagonist with low binding affinity at the orthostatic site at the CB1 receptor and an agonist at the CB2 receptor (McPartland *et al.*, 2015; Hazzah *et al.*, 2020; Shahbazi *et al.*, 2020). The specific mechanisms of action for biological effects caused by CBD remains unclear (Hazzah *et al.*, 2020; Gray & Whalley, 2020) because at least 65 molecular targets for CBD exists and its ”activity is multimodal” with the vast majority of it not being cannabinoid receptor dependent (Ibeas Bih *et al.*, 2015; Hazzah *et al.*, 2020).

References

Atakan, Z. 2012. Cannabis, a complex plant: Different compounds and different effects on individuals. Ther. Adv. Psychopharmacol. 2, 241–254 https://doi.org/10.1177/2045125312457586.

Barnham, C., & Baxter, A. 1998. Condition Factor, K, for Salmonid Fish.

Boundless. 2015. Le Chatelier’s Principle. Le Chatelier’s Princ.

Bowles, N. P., Herzig, M. X., & Shea, S. A. 2017. Recent legalization of cannabis use: Effects on sleep, health, and workplace safety. Nat. Sci. Sleep 9, 249–251 https://doi.org/10.2147/NSS.S152231.

Browne, R. G., & Weissman, A. 1981. Discriminative stimulus properties of delta 9-tetrahydrocannabinol: mechanistic studies. J. Clin. Pharmacol. 21, 227S-234S https://doi.org/10.1002/j.1552-4604.1981.tb02599.x.

Campbell, J. A. 1985. Le Châtelier’s principle, temperature effects, and entropy. J. Chem. Educ. 62, 231 https://doi.org/10.1021/ed062p231.

Chung, H., Fierro, A., & David Pessoa-Mahana, C. 2019. Cannabidiol binding and negative allosteric modulation at the cannabinoid type 1 receptor in the presence of delta-9tetrahydrocannabinol: An in Silico study. PLoS One 14, 1–18 https://doi.org/10.1371/journal.pone.0220025.

Couch, D. G., Maudslay, H., Doleman, B., Lund, J. N., & O’Sullivan, S. E. 2018. The Use of Cannabinoids in Colitis: A Systematic Review and Meta-Analysis. Inflamm. Bowel Dis. 24, 680–697 https://doi.org/10.1093/ibd/izy014.

Dempsey, P. 2021. Tilapia farming: Untapped SA market holds opportunity for growth. , 20–23.

De Briyne, N., Holmes, D., Sandler, I., Stiles, E., Szymanski, D., Moody, S., Neumann, S., & Anadón, A. 2021. Cannabis, cannabidiol oils and tetrahydrocannabinol—what do veterinarians need to know? Animals 11, 1–19 https://doi.org/10.3390/ani11030892.

Di Marzo, V. 1998. ‘Endocannabinoids’ and other fatty acid derivatives with cannabimimetic properties: Biochemistry and possible physiopathological relevance. Biochim. Biophys. Acta - Lipids Lipid Metab. 1392, 153–175 https://doi.org/10.1016/S0005-2760(98)00042-3.

Du, Z.-Y., Clouet, P., Zheng, W.-H., Degrace, P., Tian, L.-X., & Liu, Y.-J. 2006. Biochemical hepatic alterations and body lipid composition in the herbivorous grass carp (Ctenopharyngodon idella) fed high-fat diets . Br. J. Nutr. 95, 905–915 https://doi.org/10.1079/bjn20061733.

El-Sayed, A. F. M. 2006. Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials. Av. en Nutr. Acuícola VIII, 95–106.

Elmes, M. W., Kaczocha, M., Berger, W. T., Leung, K. N., Ralph, B. P., Wang, L., Sweeney, J. M., Miyauchi, J. T., Tsirka, S. E., Ojima, I., & Deutsch, D. G. 2015. Fatty acid-binding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). J. Biol. Chem. 290, 8711–8721 https://doi.org/10.1074/jbc.M114.618447.

Evans, F. J. 1991. Cannabinoids: the separation of central from peripheral effects on a structural basis. Planta Med. 57, S60-7.

Firth, D. C. 2018. Temporal and inter-species variations in the proximate and contaminant compositions of farmed mussels , Choromytilus meridionalis and Mytilus galloprovincialis , from Saldanha Bay , South Africa. , 1–123.

Food and Agriculture Organization of the United Nations. 2020. The State of World fisheries and aquaculture in review. FAO.org, Rome.

Food and Agriculture Organization of the United Nations (FAO). 2016. Oreochromis niloticus (Linnaeus, 1758). Rome.

Food and Agriculture Organization of the United Nations (FAO). 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action.

Froese, R. 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22, 241–253 https://doi.org/https://doi.org/10.1111/j.1439-0426.2006.00805.x.

Gomiero, L. M., & De Souza Braga, F. M. 2005. The condition factor of fishes from two river basins in São Paulo state, Southeast of Brazil. Acta Sci. - Biol. Sci. 27, 73–78 https://doi.org/10.4025/actascibiolsci.v27i1.1368.

Gray, R. A., & Whalley, B. J. 2020. The proposed mechanisms of action of CBD in epilepsy. Epileptic Disord. 22, 10–15 https://doi.org/10.1684/epd.2020.1135.

Hampson, A. J., Grimaldi, M., Lolic, M., Wink, D., Rosenthal, R., & Axelrod, J. 2000. Neuroprotective Antioxidants from Marijuanaa. Ann. N. Y. Acad. Sci. 899, 274–282 https://doi.org/https://doi.org/10.1111/j.1749-6632.2000.tb06193.x.

Hazzah, T., Andre, C., Richter, G., & Mcgrath, S. 2020. Cannabis in Veterinary Medicine : A Critical Review. AHVMA J. 61, 17–41.

Hinz, B., & Ramer, R. 2019. Anti-tumour actions of cannabinoids. Br. J. Pharmacol. 176, 1384–1394 https://doi.org/10.1111/bph.14426.

Hishamunda, N., & Ridler, N. B. 2006. Commercial aquaculture in Southeast Asia: Some policy lessons. Food Policy 31, 401–414 https://doi.org/10.1016/j.foodpol.2005.12.004.

Hossain, M. Y., Ahmed, Z. F., Leunda, P. M., Jasmine, S., Oscoz, J., Miranda, R., & Ohtomi, J. 2006. Condition, length-weight and length-length relationships of the Asian striped catfish Mystus vittatus (Bloch, 1794) (Siluriformes: Bagridae) in the Mathabhanga River, southwestern Bangladesh. J. Appl. Ichthyol. 22, 304–307 https://doi.org/10.1111/j.1439-0426.2006.00803.x.

Ibeas Bih, C., Chen, T., Nunn, A. V. W., Bazelot, M., Dallas, M., & Whalley, B. J. 2015. Molecular Targets of Cannabidiol in Neurological Disorders. Neurotherapeutics 12, 699–730 https://doi.org/10.1007/s13311-015-0377-3.

James, N. 2012. Tilapia farming can be done in SA. Farmer's Wkly.

James, N. 2019. Aquaculture in Africa: the successes and failures. Farmer's Wkly.

Jones, R. E., Petrell, R. J., & Pauly, D. 1999. Using modified length-weight relationships to assess the condition of fish. Aquac. Eng. 20, 261–276 https://doi.org/10.1016/S0144-8609(99)00020-5.

Kentucky State University Aquaculture. 2015. Tilapia. Tilapia.

Khallaf, E. A., Galal, M., & Authman, M. 2003. The biology of Oreochromis niloticus in a polluted canal. Ecotoxicology 12, 405–416 https://doi.org/10.1023/a:1026156222685.

Komorowski, J., & Stepień, H. 2007. [The role of the endocannabinoid system in the regulation of endocrine function and in the control of energy balance in humans]. Postepy Hig. Med. Dosw. (Online) 61, 99–105.

Le Cren, E. D. 1951. The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in the Perch (Perca fluviatilis). J. Anim. Ecol. 20, 201–219 https://doi.org/10.2307/1540.

Le Foll, B., Trigo, J. M., Sharkey, K. A., & Strat, Y. Le. 2013. Cannabis and Δ9-tetrahydrocannabinol (THC) for weight loss? Med. Hypotheses 80, 564–567 https://doi.org/10.1016/j.mehy.2013.01.019.

Luckhoff, P. D. 2005. Application of the condition factor in the production of African Sharptooth Catfish Clarias gariepinus.

McPartland, J. M., Agraval, J., Gleeson, D., Heasman, K., & Glass, M. 2006a. Cannabinoid receptors in invertebrates. J. Evol. Biol. 19, 366–373 https://doi.org/10.1111/j.1420-9101.2005.01028.x.

McPartland, J. M., Duncan, M., Di Marzo, V., & Pertwee, R. G. 2015. Are cannabidiol and Δ9-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br. J. Pharmacol. 172, 737–753 https://doi.org/10.1111/bph.12944.

McPartland, J. M., Matias, I., Di Marzo, V., & Glass, M. 2006b. Evolutionary origins of the endocannabinoid system. Gene 370, 64–74 https://doi.org/10.1016/j.gene.2005.11.004.

Morello, G., Imperatore, R., Palomba, L., Finelli, C., Labruna, G., Pasanisi, F., Sacchetti, L., Buono, L., Piscitelli, F., Orlando, P., Di Marzo, V., & Cristino, L. 2016. Orexin-A represses satiety-inducing POMC neurons and contributes to obesity via stimulation of endocannabinoid signaling. Proc. Natl. Acad. Sci. U. S. A. 113, 4759–4764 https://doi.org/10.1073/pnas.1521304113.

Muchlisin, Z. A., Musman, M., & Siti Azizah, M. N. 2010. Length-weight relationships and condition factors of two threatened fishes, Rasbora tawarensis and Poropuntius tawarensis, endemic to Lake Laut Tawar, Aceh Province, Indonesia. J. Appl. Ichthyol. 26, 949–953 https://doi.org/10.1111/j.1439-0426.2010.01524.x.

Naftali, T., Bar-Lev Schleider, L., Dotan, I., Lansky, E. P., Sklerovsky Benjaminov, F., & Konikoff, F. M. 2013. Cannabis induces a clinical response in patients with crohn's disease: A prospective placebo-controlled study. Clin. Gastroenterol. Hepatol. 11, 1276-1280.e1 https://doi.org/10.1016/j.cgh.2013.04.034.

Nash, R. D. M., Valencia, A. H., & Geffen, A. J. 2006. The origin of Fulton's condition factor - Setting the record straight. Fisheries 31, 236–238.

Omogoriola, H., Williams, A., Adegbile, O., Olakolu, F., Ukaonu, S., & Myade, E. 2011. Length- weight relationships, condition factor (K) and relative condition factor (Kn) of Sparids, Dentex congoensis

(Maul, 1954) and Dentex angolensis (Maul and Poll, 1953), in Nigerian coastal water. Int. J. Biol. Chem. Sci. 5 https://doi.org/10.4314/ijbcs.v5i2.72147.

Pauly, D. 1984. Fish population dynamics in tropical waters: A manual for use with programmable calculators. 143rd ed. International Center for Living Aquatic Resources Management, Manila, Philippines.

Ricker, W. E. 1975. Computation and Interpretation of Biological Statistics of Fish Populations (JC Stevensons, J Watson, RH Wigmore, & JM Reinhart, Eds.). Department of the Environment, Fisheries and Marine Service, Ottawa.

Russo, E. B. 2007. History of Cannabis and Its Preparations in Saga, Science, and Sobriquet. Chem. Biodivers. 4, 1614–1648 https://doi.org/10.1002/cbdv.200790144.

Saoud, I., Babikian, J., Nasser, N., & Monzer, S. 2018. Effect of cannabis oil on growth performance, haematology and metabolism of Nile Tilapia Oreochromis niloticus. Aquac. Res. 49, 809–815 https://doi.org/10.1111/are.13512.

Shahbazi, F., Grandi, V., Banerjee, A., & Trant, J. F. 2020. Cannabinoids and Cannabinoid Receptors: The Story so Far. iScience 23, 101301 https://doi.org/10.1016/j.isci.2020.101301.

Silver, R. J. 2019. The Endocannabinoid System of Animals. Animals 9, 686 https://doi.org/10.3390/ani9090686.

Small, E. 2017. Cannabis: A complete guide. Taylor & Francis Group, LLC, Boca Raton, Florida.

South African Department: Trade and Industry. 2020. Investing in South Africa ' s Aquaculture Sector - A future growth sector to be unlocked. South Africa - Aquac. Factsheet 1, 2.

Tarkan, A. S., Gaygusuz, O., Acipinar, H., Gursoy, C., & Ozulug, M. 2006. Length-weight relationship of fishes from the Marmara region (NW-Turkey). J. Appl. Ichthyol. 22, 271–273 https://doi.org/10.1111/j.1439-0426.2006.00711.x.

The Department of Agriculture Forestry and Fisheries. 2015. Aquaculture year book South Africa.

The Department of Agriculture Forestry and Fisheries. 2016. Aquaculture Yearbook 2016 South Africa. Cape Town.

The Department of Agriculture Forestry and Fisheries (DAFF). 2018. Nile and Mozambique Tilapia Feasibility Study. Nile Mozambique Tilapia Feasibility Study 9, 1–92.

Tomida, I., Azuara-Blanco, A., House, H., Flint, M., Pertwee, R. G., & Robson, P. J. 2006. Effect of sublingual application of cannabinoids on intraocular pressure: a pilot study. J. Glaucoma 15, 349–353 https://doi.org/10.1097/01.ijg.0000212260.04488.60.

Tveteras, R. 2016. Global Fish Production Data & Analysis.in Global Fish Production Data & Analysis.

United Nations. 2015. Transforming our world: the 2030 Agenda for Sustainable Development.

United Nations Development Programme. 2015. Sustainable Development Goals.

United States Environmental Protection Agency (EPA). 2019. Ammonia. CADDIS 2.

Upton, R., Craker, L., Elsohly, M. A., Romm, A., Russo, E., Sexton, M., Marcu, J., & Swisher, D. 2014. American Herbal Pharmacopoeia (R Upton, L Craker, MA Elsohly, A Romm, E Russo, & M Sexton, Eds.).

VanDolah, H. J., Bauer, B. A., & Mauck, K. F. 2019. Clinicians' Guide to Cannabidiol and Hemp Oils. Mayo Clin. Proc. 94, 1840–1851 https://doi.org/10.1016/j.mayocp.2019.01.003.

Wang, M., Wang, Y. H., Avula, B., Radwan, M. M., Wanas, A. S., Van Antwerp, J., Parcher, J. F., Elsohly, M. A., & Khan, I. A. 2016. Decarboxylation Study of Acidic Cannabinoids: A Novel Approach Using Ultra-High-Performance Supercritical Fluid Chromatography/Photodiode Array-Mass Spectrometry. Cannabis Cannabinoid Res. 1, 262–271 https://doi.org/10.1089/can.2016.0020.

Westlab. 2017. How Does Temperature Affect pH?

Williams, S. J., Hartley, J. P. R., & Graham, J. D. P. 1976. Bronchodilator effect of Δ1 tetrahydrocannabinol administered by aerosol to asthmatic patients. Thorax 31, 720–723 https://doi.org/10.1136/thx.31.6.720.

Wood, L. 2019. Analysis on the World's $36.9 Billion Cannabis Market, 2019-2024: Data on both Medicinal & Recreational End-uses. Bus. Wire.

Wurts, W. a. 1992. Pond pH and Ammonia Toxicity. World Aquac. 34, 20–21.

Zar, J. H. 1984. Biostatistical Analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs.

Zuardi, A. W. 2008. Cannabidiol : from an inactive cannabinoid to a drug with wide spectrum of action Canabidiol : de um canabinóide inativo a uma droga com amplo espectro de ação. Rev. Bras. Psiquiatr. 30, 271–280 https://doi.org/10.1590/S1516-44462008000300015.

Chapter 3 Growth parameters for three treatments with CBD oil given at different concentrations

3.1. Abstract

Industrialised intensive production systems are becoming more prevalent in the aquaculture industry, which indirectly increase the pressure on fish resources due to the inclusion of fish meal in fish diets. Alternative protein feeds for the replacement of fishmeal are available, but diet palatability is compromised when alternative protein sources are used. This study investigated CBD as an additive that can potentially cause an increase in appetite, indirectly subjecting fish to eat alternative protein feeds. A commercial Tilapia feed was covered with three different concentrations of CBD; 20%, 40% and 60% and was fed to Tilapia for 10 weeks. Biweekly, fish weight and length were measured and at termination, survival, growth parameters, and feed conversion ratio (FCR) were assessed. At termination, CBD was found to have not caused significant increases among treatments in growth parameters, specific growth rate (SGR) and feed conversion ratio (FCR). However, significant difference for Fulton's condition index was reported among the treatments. Water quality parameters like water temperature, pH and dissolved oxygen (DO) were all within the optimum ranges for *O. niloticus*. CBD therefore did not affect the feeding behaviour or appetite of *O. niloticus*.

3.2. Introduction

Aquaculture is the fastest-growing food production sector in the world (Food and Agriculture Organization of the United Nations, 2020), however, the biggest hurdle in the way is the price of fish feed, being as much as 60% of the production costs (El-Sayed, 2006), thus affecting profitability of the industry in other places.

One of the most expensive components of a fish diet is protein, which is required for growth and maintenance functions. The most common source of protein in fish diets is fish meal (FM). According to Madage *et al.* (2015), the practice of using FM in animal diets is an unsustainable practice. Yet, Tacon & Metian (2015) and Fry et al. (2018) suggest that aquaculture feed has a significant inclusion of ingredients made from wild-caught fish. This dismantle the argument for aquaculture being more sustainable if its utilising ingredients derived from unsustainable sources like wild-caught fish.

The replacement of FM with alternative proteins such as soybean meal (SBM) and processed canola meal (PCM), does not result in comparable growth rates that are achieved when FM is

included in fish diets (Al-Ghanim *et al.*, 2017; Egerton *et al.*, 2020; Mohammadi *et al.*, 2020). According to Djissou *et al.* (2016), the total replacement of FM generally leads to a decrease in feed intake and efficiency, and a decrease in growth performance, as a result of unpalatability of FM alternatives, which can be a cause for the decreased feed intake. To reduce the limitations associated with the abovementioned side effect of FM alternative proteins, the 'munchies' effect caused by cannabinoids found in *Cannabis sativa* can be investigated to determine its effects on growth performance.

Cannabis sativa, also known as hemp, marijuana, pot, hashish and weed is a herbaceous plant species belonging to the Cannabaceae family that originated from Central Asia (Zuardi, 2008). According to Russo (2007) , secondary products of the *C. sativa* plant has many beneficial uses for "chronic pain, spasticity, seizure disorders, cancer, etc.". According to Saoud *et al.* (2018) and Russo (2007), these ailments are ill understood and not a lot of evidence exist to understand the role *C. sativa* play.

Cannabis sativa consist of more than 400 chemical compounds and more than 60 cannabinoids which can be found in resin within flowers, leaves and seeds (Atakan, 2012). Cannabidiol (CBD) is but one of these cannabinoids and is the second most studied cannabinoid to delta-9-tetrahydrocannabinol (THC). According to Ignatowska-Jankowska *et al.* (2011), there are few studies that have focused on studying the potential effects of CBD on feeding behaviour of rats and the effects of chronic administration of CBD on body weight (Wiley *et al.*, 2005; Riedel *et al.*, 2009; Izzo *et al.*, 2009). Saoud *et al.* (2018) investigated the effects of CBD as a treatment on feeding behaviour of Nile Tilapia and found no significant effects. However, Saoud *et al.* (2018) suggested that because a variety of cannabinoids were involved and the inability to identify which cannabinoid caused which effect, an isolated focus should be given to establish the effects of one cannabinoid like CBD.

Therefore, the aim of this chapter was to assess the effect of CBD on the growth performance of *O.* niloticus. After carps, *Oreochromis niloticus* also known as Nile Tilapia, is the second most cultured fish in the world (Azaza *et al.*, 2008; Abdelhadi, 2011; Saoud *et al.*, 2018). *O. niloticus* are increasingly being cultured as its popularity increases globally. However, an increase in *O. niloticus* rearing means an increase in feed requirements. This can add to the already strained supplies of fishmeal. The present study was designed to test whether CBD could be used as a top cover for fish feed to improve ingestion and assimilation, thus feed intake. Accordingly, the effects of different CBD concentrations (0 mg/kg, 20 mg/kg, 40 mg/kg, and 60 mg/kg) on growth parameters of juvenile *O. niloticus* were investigated.

3.3. Materials and Methods

Ethical approval for the study was obtained from the Research Ethics Committee: Animal Care and Use (ACU-2020-14573).

3.3.1. Experiment location

All feeding experiments relating to the growth experiment were performed at the facilities of the Aquaculture Division of Stellenbosch University on the Welgevallen Experimental Farm. The experimental recirculating aquaculture system (RAS) was situated in one of the plastic growth tunnels of the Agronomy Department.

3.3.2. Experimental animals and Husbandry

Nile Tilapia (*Oreochromis niloticus*) were used as experimental fish in this study. The stocking density were 30 fish per tank and six tanks per treatment, connected to a biological filter also acting as the header tank, a three-tank settling mechanical filter as well as a two-tank sump. Each treatment had 180 fish and in total 720 fish were used. Fish were reared in 28 circular fibre glass (D = 150 cm; H = 30 cm) tanks with the volume capacity of 350 L each. Fish were fed a commercial Tilapia diet at a feeding regime recommended by the manufacturer, 5% body weight, three times per day.

Water in the system was aerated using a regenerative blower and submerged 30 mm air stones. The RAS-setup was in an agricultural plastic growth tunnel that is influenced by ambient high and low temperatures for the day. However, temperature ranged between 25 - 34 ˚C on average at feeding times. Temperature, pH, and dissolved oxygen were measured before feeding occurred. ammonia, nitrite, and nitrate nitrogen were measured fortnightly.

3.3.3. Experimental design and treatments

At the start of the experimental trial, Tilapia of similar sizes, 15 – 17 g was placed in the 24 tanks. The fish were offered; a commercial feed with a 40% crude protein, and an 8.6% fat commercial feed at 5% total average BW three times a day (AVI Products (Pty) Ltd., Reg. No. 2001/015923/07, Cato Ridge, Kwazulu-Natal, South Africa).

The control and three treatments (T20, T40 and T60) were assigned according to each of the four rows of tanks, illustrated in Figure **3.1**. Each of the three treatments and control group were assigned to six of the 24 tanks, each. The Tilapia in treatments 20, 40 and 60 were offered a prepared diet and one unprepared diet functioning as the control. The prepared diet was fed at 5% BW of the average fish weight per tank in each of the treatments at 10:00, 13:00 and 16:00.

The fish was offered the above-mentioned diets for 10 weeks. Feeding started at 10 am as the water temperature before 10 am was below the optimum water temperature required. Literature indicates that feeding outside the optimum water temperature ranges leads to an increased FCR and contributes to bad water quality.

3.3.4. Preparation and storage of treatment diets

CBD isolates were derived from hemp (*Cannabis sativa L.)* mixed with industrial hemp seed oil extracted by means of cold press extraction. The oil was obtained from a commercial seller, Milagro. The product come in a range of different inclusion levels of CBD that is pre-determined, the highest CBD inclusion level product were chosen i.e., 2000 mg per 30 ml. The CBD extract were mixed with virgin coconut oil in a ratio of 1-part CBD oil to 500 parts coconut oil (1:500) i.e., 0.1 ml CBD oil for every 50 ml of virgin coconut oil per kg of feed because the amount of CBD oil extract used for each treatment would not have been able to cover all pellets equally. The virgin coconut oil did not just serve as a medium to dilute the CBD concentration, but also one to ensure that the predetermined CBD concentration can be well covered over the pellets. The oil mixture was then used as a top cover for the Tilapia starter 2mm feed and allowed to dry for ten minutes (Table 3.1 obtained from AVI Products (AVI Products (Pty) Ltd., Reg. No. 2001/015923/07, Cato Ridge, Kwazulu-Natal, South Africa).

The fish were fed a feed consisting of a floating pellet that ensured fish with increased appetite could feed on the uneaten feed and do not lose weight as result of increased metabolism described by (Saoud *et al.*, 2018).

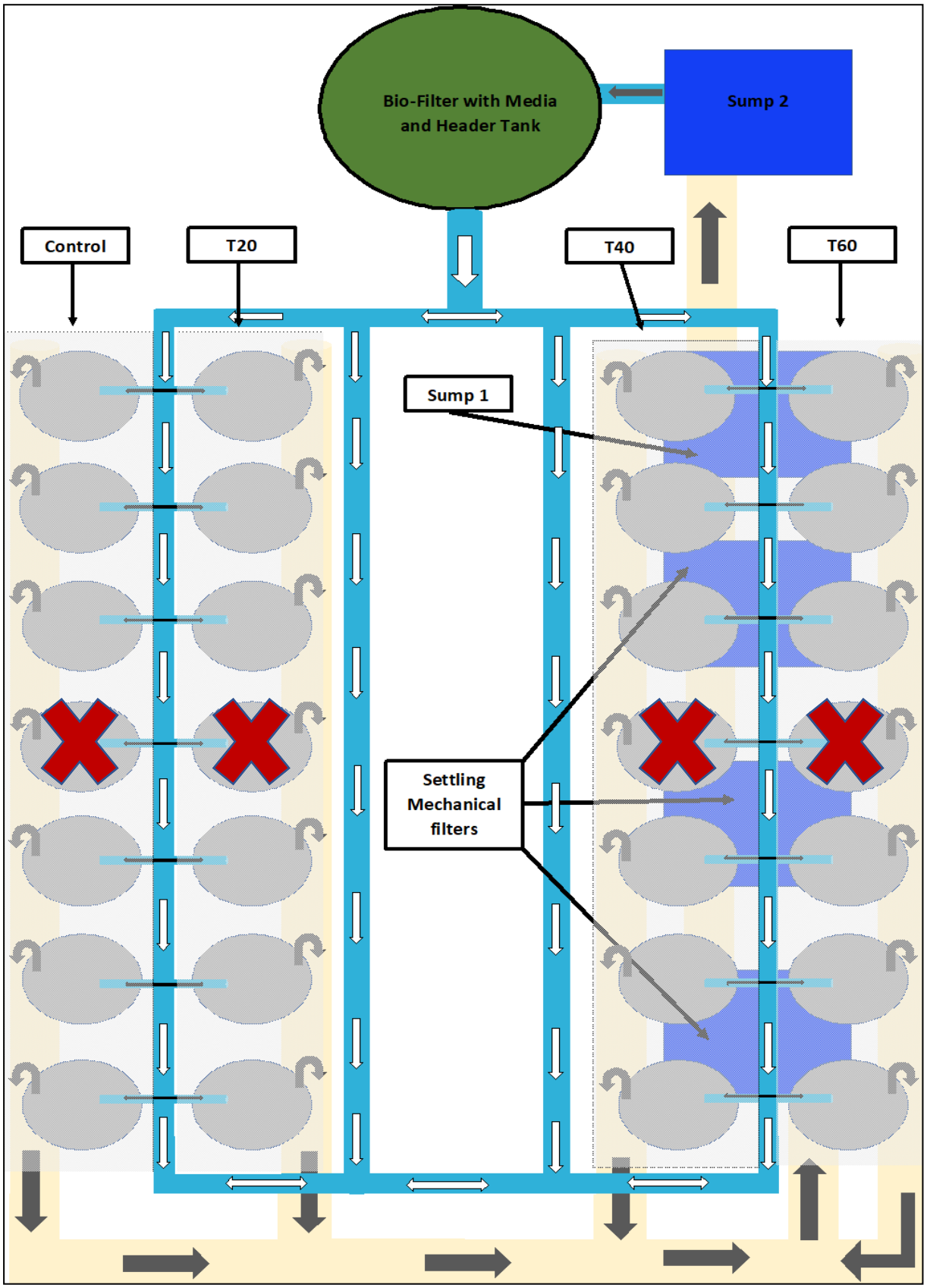


Figure 3.1 Schematic of system design (Jocelyn & Juries, 2020). Tanks marked with X, are fish selected from each treatment for haematology.

From here onwards, T20, T40 and T60 refers to treatments and the numeric values refers to the CBD concentrations (20, 40, and 60 mg/kg) used in these treatments.

The T20 diet oil was prepared by mixing 9 ml of CBD oil (600 mg predetermined by the manufacturer) with 1 500 ml of virgin coconut oil. This oil mixture was then used as a top cover over 30 kg of feed. The oil was mixed using a Kenwood stand mixer at slow speed for about 10 minutes to ensure the coconut oil – CBD oil mixture was thoroughly mixed.

The T40 diet oil was prepared by mixing 18 ml of CBD oil extract (1200 mg predetermined by the manufacturer) with 3 000 ml of virgin coconut oil. This oil mixture was used as a top cover for 30 kg of feed. The same mixing and storage protocol followed in the preparation of diet T20 was followed.

The T60 diet oil was prepared by mixing 27 ml of CBD oil extract (1800 mg, predetermined by the manufacturer) with 3 600 ml of virgin coconut oil. This oil mixture was used as a top cover for 30 kg of feed. The same mixing and storage protocol as in T20 and T40 was followed.

A total of 90 kg of Tilapia grower 2mm feed was covered with the coconut oil – CBD mixture. To ensure that all pellets were evenly coated with the oil, batches of 10 kg was used at a time. A MacAdams commercial dough mixer was used to mix pellets with oil and to ensure all pellets were evenly covered with oil. The oil on the coated pellets was then allowed to solidify in a temperature-controlled room at 20˚C after which it was stored in the fish feed storage facility at Welgevallen Experimental Farm. The same method was used when the control group's feed was covered with just coconut oil.

After the diet preparation of each treatment group was completed, all utensils and equipment used in the preparation was washed and wiped with 70% ethanol to ensure sterility and to prevent contamination.

Table 3.1 Feed formulation of Commercial Tilapia feed for each feed type adapted from AVI Feeds (2020).

Nutritional composition of Tilapia feeds

Nutrient		Tilapia Fry (No.0 powder and No.1,2,3 crumble)	Tilapia Starter 2mm	Tilapia Grower 3mm	Tilapia Finisher 5mm
Metabolisable Energy	MJ/kg	12|8	12|9	12|7	13|3
Digestible Energy	MJ/kg	14|8	14|9	14|7	14|8
Crude Protein	g/kg	450	400	350	300
Lysine	g/kg	25|2	21|4	18|6	15|3
Methionine	g/kg	9|5	7|5	6|1	5|6
T.S.A.A.	g/kg	16|9	14|3	12|4	11|5
Isoleucine	g/kg	19|3	17|3	15|4	13|7
Tryptophan	g/kg	4|3	4	3|7	3|1
Threonine	g/kg	18|6	16|2	14|1	12|8
Valine	g/kg	24|1	21	18|3	16|8
Arginine	g/kg	28|7	26|2	23|4	21|2
Fat	g/kg	85	86	74	77
Fibre	g/kg	23	29	32	28
Ash	g/kg	93	75	62	55
Calcium	g/kg	15|7	12|8	10|6	10|2
Total Phosphorus	g/kg	12|4	11|6	9|4	8|8
Sodium	g/kg	3|2	2|6	2|4	2

Sizes of the Crumbles	
#0	<500µm
#1	500-750µm
#2	750-1500µm
#3	1500-2500µm

3.4. Data recorded

Individual weight, and length was recorded for each fish at the start of the feeding trial and bi-weekly thereafter. Water temperature was constantly measured and monitored with an indoor-outdoor min/max thermometer, with the outdoor probe in the sump. DO was measured once a day in the early afternoon feeding with an Oxyguard probe. pH was also measured once a day in the early afternoon feeding with a Sega waterproof pH pen. Ammonia, Nitrite and Nitrate were measured weekly with a JBL Pro-Aqua Ammonium NH_4 Test, JBL Pro-Aqua Nitrite NO_2 Test and a JBL Pro-Aqua Nitrate NO_3 test, respectively. Water quality parameters were tested to ensure fish wellbeing and optimum growth environment for the fish as well as to ascertain consistent readings across experimental tanks.

The following equations were used to calculate the Specific Individual Growth Rate ($SGR_{Individual}$) (%/d), Absolute Biomass Growth Rate ($AGR_{Biomass}$), Feed Conversion Ratio (FCR) and the Condition factor (K).

$$SGR_{Individual} = \frac{ln(IWf) - ln\,(IWi)}{nt} \times 100 \quad \textbf{3.1}$$

Where $SGR_{Individual}$ = Specific Growth Rate for average individual wight (%/d)
Ln (IWf) = Natural logarithm of final average individual weight (g)
Ln (IWi) = Natural logarithm of initial average individual weight (g)
nt = Number of rearing days (d)

$$AGR_{Biomass} = Bf - Bi \quad \textbf{3.2}$$

Where $AGR_{Biomass}$ = Absolute Growth Rate for biomass (g)
Bf = Final biomass (g)
Bi = Initial biomass (g)

$$FCR = \frac{TFG}{AGR_{Biomass}} \quad \textbf{3.3}$$

Where FCR = Feed Conversion Ratio (g/g)
TFG = Total Feed Given (g)
$AGR_{Biomass}$ = Absolute Growth Rate for biomass (g)

$$K = \frac{100W}{L^b} \quad \textbf{3.4}$$

Where K = Condition Factor (Pauly, 1984; Gomiero & De Souza Braga, 2005)
W = Final Body Weight (FBW) in gram (g)
L = Total Length of fish in centimetres (cm)
b = exponent of the length-weight equation

$$W = aL^b \text{ (Pauly, 1984)} \quad \textbf{3.5}$$

Where W = Weight of fish (g)
a = exponent describing the rate of change of weight with length (intercept)
L = Total Length of fish in centimetres (cm)
b = Weight at unit length (slope)

$$log\,W = b\,log\,L + log\,a \text{ (Zar, 1984)} \quad \textbf{3.6}$$

Where W = Weight of fish (g)

b = Weight at unit length (slope)

L = Total Length of fish in centimetres (cm)

a = exponent describing the rate of change of weight with length (intercept)

3.5. Statistical analysis

The latest version of R (R Core Team, 2020). (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.) was used where ANOVA were used to analyse data together with test assumptions (normality and homoscedasticity) and Tukey (HSD).

3.6. Results

3.6.1. Growth experiment

Juvenile *Oreochromis niloticus* survived being subjected to all treatments and grew in weight and length with growth not being significant among treatments at a significance level of 0.05 (Table 3.**3**, Figure 3.2). Average final body weight (FWB) among treatments did not show significant differences among treatments. There are noticeable differences among the treatment groups for specific growth rate (SGR) (Equation 2.1), however it does not differ significantly statistically ($p > 0.05$) (Figure 3.3). As can be seen in Table 3.3, average total length at harvest (TL) also seems to have increased among treatments although the control group had a slightly higher average TL at harvest than T20. The feed conversion ratio (FCR) (Equation 3.3) among treatments did not show a significant difference among treatments. The condition factor (K) of the fish were significantly different among treatments (Table 3.5). The b exponent of the body condition equation for each tank were calculated with averages of the treatments subjected to a t-test to establish significance. The b exponent for each treatment were significantly different from each other (Table 3.4) but did not deviate significantly from $b = 3$ ($p = 0.1034$) that's given by Fulton's body condition for isometric growth.

Table 3.2 Survival (S), initial body weight (IBW), final body weight (FBW), total length (TL) at harvest, and feed conversion ratio (FCR) of juvenile *Oreochroms niloticus* on different diets.

Treatment	S (%)	IBW ± SE (g)	Growth rate ± SE (g/day)	FBW ± SE (g)	TL (cm)	FCR
Control	100	17.49 ± 0.37	1.18 ± 0.12	99.69 ± 0.21	16.81	1.90
T20	100	15.63 ± 0.38	1.19 ± 0.12	84.88 ± 0.28	16.79	1.77
T40	100	15.85 ± 0.35	1.22 ± 0.12	86.93 ± 0.23	17.06	1.72
T60	100	17.18 ± 0.36	1.28 ± 0.12	91.30 ± 0.26	17.52	1.87

Table 3.3 Exponent b for body condition equation and Body condition factor (K) (mean ± SE) for Nile Tilapia that received CBD oil.

Treatment inclusion level (mg/kg)					P - value
	0	20	40	60	
Exponent b	2.88 ± 0.16	2.71 ± 0.43	2.85 ± 0.11	2.96 ± 0.10	1.321×10^{-5}
Body condition factor (K)	3.03 ± 0.045	4.59 ± 0.11	3.05 ± 0.03	2.17 ± 0.03	0.007789

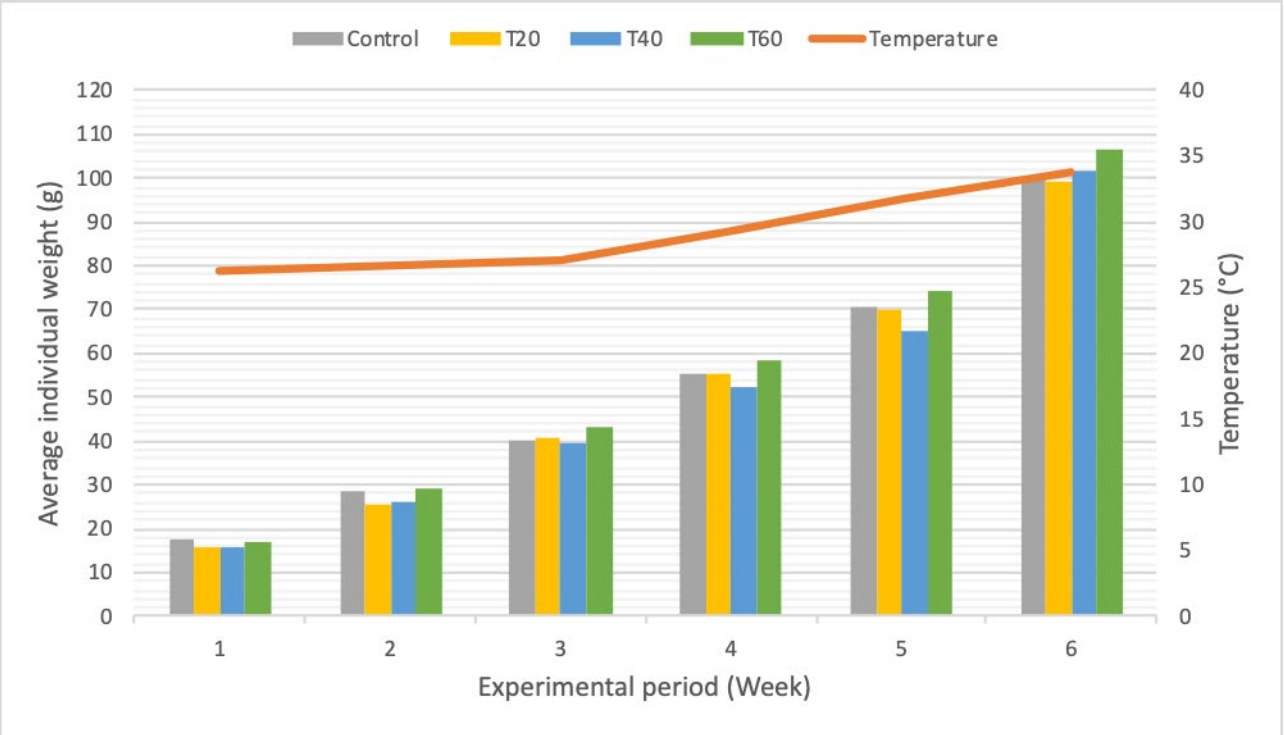


Figure 3.2 Growth in average individual body weight per tank (g) against Temperature (°C) over 10 weeks *O. niloticus*, offered different treatment diets.

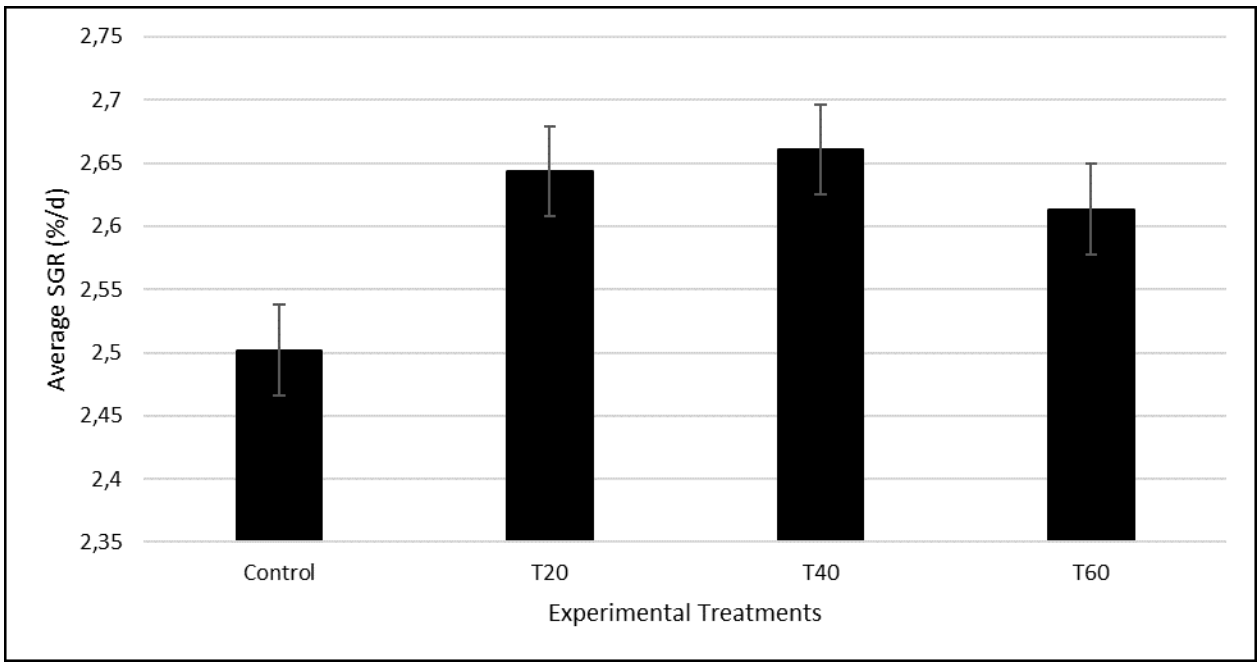


Figure 3.3 Average specific growth rates of juvenile *O. niloticus* during the experimental period. No significant differences at α = 0.05.

The average water temperature across the production system for the first five weeks averaged between 26-28˚C. Week 6 and 7 averaged just above 30℃ while week 8 – 10 averaged just under 35˚C (Figure 3.4). The pH across the production system for week 1 – 2 averaged just over 7.00. During the remainder of the trial period from week 3 to 10 the pH across the production system averaged between 7.60 – 7.80 as the biofilter matured, converting nitrite into nitrate (**Figure 3.5**). The dissolved oxygen (DO) on averaged followed a downward trend as the trial progressed. Apart from a slight increase in the DO level during week 2, the DO level across the production system decreased weekly Figure 3.6, averaging at 5.63 mg/l with the lowest recorded DO at 3.86 mg/l and highest at 7.30 mg/l. The ammonium concentration on average was lower than 0.05 mg/L with the highest ammonium concentration measured at 0.20 mg/L in week 8. The nitrate concentration on average across the 10-week experimental period was 2.60 mg/L with 1.00 and 5.00 mg/L being the lowest and highest, respectively. The nitrite concentration on average across the experimental period was 0.18 mg/L with 0.05 and 0.70 being the lowest and highest measurements, respectively Table 3.3).

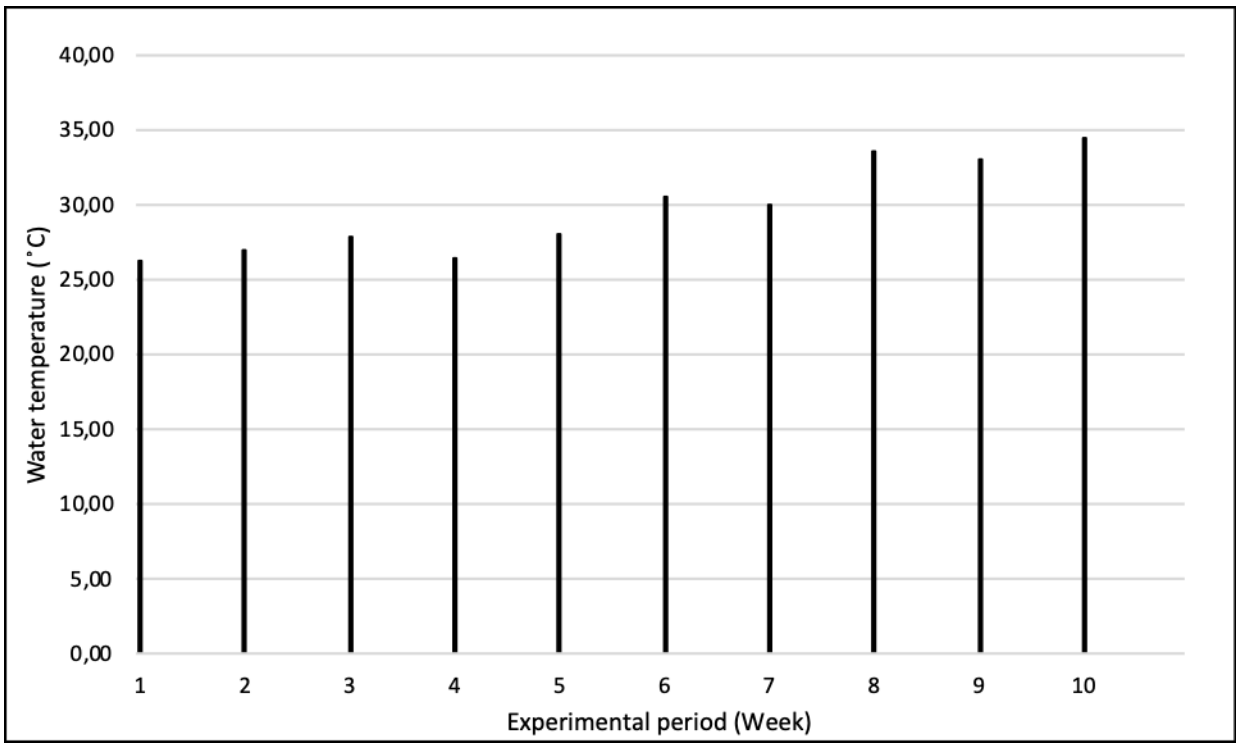


Figure 3.4 Average water temperature (℃) for the duration of the experimental period of 10 weeks.

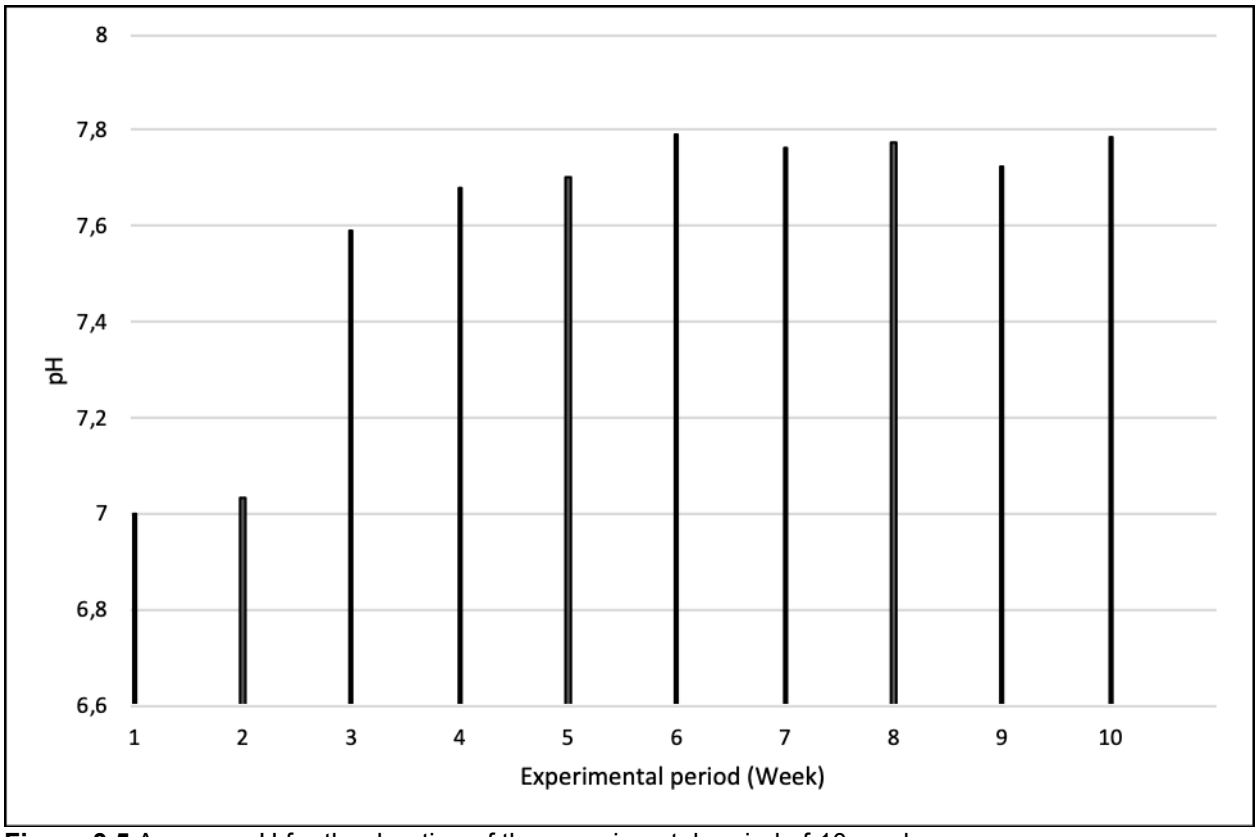


Figure 3.5 Average pH for the duration of the experimental period of 10 weeks.

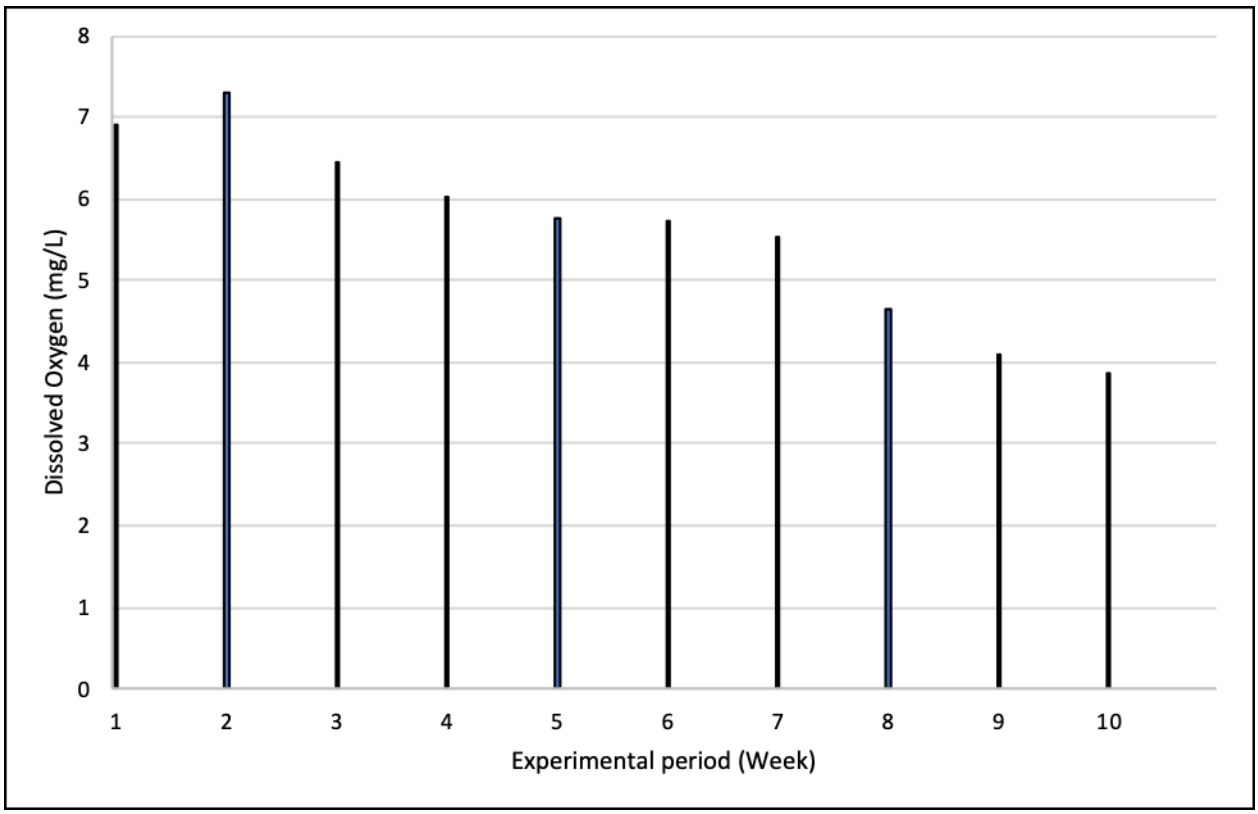


Figure 3.6 Average dissolved oxygen (DO) (mg/L) for the duration of the experimental period of 10t weeks.

3.7. Discussion

3.7.1. Effect of CBD extract on growth

Average body weight was not influenced by any of the treatments (P≥0.05). No other studies could be found where CBD isolate were investigated. This is contrary to the findings by Saoud *et al.* (2018) that found that cannabis and hemp caused reduced body weight with no significant differences between cannabis and hemp for body weight, however a significant difference between the control which was a soy-based diet and both cannabis and hemp were found.

It was found that dietary THC caused growth reduction in fish in comparison with diets containing hemp extract and the control (Saoud *et al.*, 2018). Ignatowska-Jankowska *et al.* (2011) suggested that the endocannabinoid system plays a role in body weight, feed intake and energy balance regulation and much focus has been placed on the topic for potential links to “obesity, eating disorders and metabolic diseases” (Di Marzo & Matias, 2005; Matias *et al.*, 2006; Di Marzo, 2008; Pertwee, 2009). Ignatowska-Jankowska *et al.* (2011), conducted a study on male Wistar rats by injecting them intraperitoneally with different concentrations of CBD, where they found that CBD can change body weight gain via the CB2 receptor that is one of the type of receptors in the endocannabinoid system. In the latter study they found that CBD decreased body weight gain in rats and so also Saoud *et al.* (2018) found that in comparison with the control the hemp extract

yielded a significantly lower body weight. Even though the results are contradictory to what was found in the present study, the administration of the CBD, in Ignatowska-Jankowska (2011), Saoud *et al.* (2018) and the present study, were all different. Intraperitoneal injection is when the substance, in this case CBD, gets injected into the body cavity (Wang *et al.*, 2016a). Saoud *et al.* (2018) made a diet containing products that contain trace amounts of CBD. The present study did not design and manufacture a diet, but merely covered a commercial feed with CBD isolate extract. The CBD was not subjected to heat, or any elements involved in the feed extraction process that could have affected the integrity of the CBD molecules.

In the present study hemp oil extract that contains CBD isolate was used and not just the hemp oil extract alone. The contrasting results with regards to growth between Saoud *et al.* (2018) and the present study could possibly be as a result of the CBD concentration administered. Saoud *et al.* (2018) used an industrial hemp concentration of 21.3 g/Kg. According to Kleinhenz *et al.* (2020), industrial hemp contains about 262 µg/g CBD in its seed heads. This equates to a possibility of 5.56 mg/kg CBD in the industrial hemp diet that Saoud and colleagues used in their study since nutrient composition as well as cannabinoid concentration of the plant parts of *C. sativa* were unknown (Kleinhenz *et al.*, 2020). The industrial hemp used in Saoud *et al.* (2018) more or less contains the same CBD concentration of that studied by Kleinhenz *et al.* (2020).

Three CBD concentrations as treatments (T20 – 20 mg/kg, T40 – 40 mg/kg, T60 – 60 mg/kg) against the control was studied that's much higher than that of Saoud *et al.* (2018). Even though its much higher than the diet inclusion level of CBD in the study by Saoud *et al.* (2018), it still did not show significant results.

According to Pertwee (2004), both CBD and THC has the ability to slow gastrointestinal motility, THC more so than CBD. The argument and anecdotal information that indicate that THC and to a certain extend CBD, increase metabolic rate, could have been explored by feeding *ad libitum* (Saoud *et al.*, 2018). Contrasting to this, a study by Shi *et al.* (2017) showed similar results ($P>0.05$) in a study with Atlantic salmon with free access to self-feeders and a time-restricted self-feeder. According to Saoud *et al.* (2018) even if the anecdotal information about increased metabolic rate in humans could be repeated in the study conducted by Saoud *et al.* (2018) and the present study, the FCR that it would have yielded would not have been worth the input costs that comes with increased feeding and would not have been have been economically viable and profitable to farmers.

3.7.2. Body condition

The body condition factor and the weight-length relationship, , respectively, of fish is an indicator of its health, well-being and nutritional status (Froese, 2006; Joscelyne, 2020). According to Ricker (1975), Nash *et al.* (2006) and Omogoriola *et al.* (2011), a higher condition factor is desired as this is seen as an indicator of good health, well-being and nutrition

The b-value suggests what type of growth, isometric or allometric, were experienced by fish. A b = 3 is an indication of isometric growth and any deviation from that is allometric growth (Ricker, 1975). Isometric growth gets described by Ricker (1975) and Omogoriola *et al.* (2011) as a uniform increase in body weight with an increase in unit of length. The isometric b-value is according to Jones *et al.* (1999) an assumption that's made by Fulton's condition factor. This assumption by Fulton's condition factor, according to Froese (2006) is not correct at all times as b-values not equivalent to three ($b \neq 3$) results in allometric growth. A b-value greater or smaller than 3 are known as positive or negative allometric growth, respectively (Omogoriola *et al.*, 2011). Positive allometric growth is an indication that the fish grows faster in weight than in length and these fish normally exhibit a body shape that's more round (Joscelyne, 2020). Negative allometric growth is the exact opposite of positive allometric growth, where the fish grows faster in length than in weight, making the fish appear to be more slender (Joscelyne, 2020). The b-value is not an absolute constant value that's affected only by a singular factor, it is known that factors like age, sex, season, maturation, fullness of the gut, feed, fat reserves and musculature can also play a role in the type of growth experienced by fish (Le Cren, 1951; Barnham & Baxter, 1998; Khallaf *et al.*, 2003; Luckhoff, 2005; Hossain *et al.*, 2006; Tarkan *et al.*, 2006; Froese, 2006; Muchlisin *et al.*, 2010; Joscelyne, 2020)

In the present study the b-values of treatments differed significantly from one another ($p < 0.05$) with all b-values indicating negative allometric growth ($b<3$). The data presented in Table 3.4 indicates that as the CBD concentration increased across treatments, the b-values also increased, moving closer to isometric growth. The b-values obtained in the present study is slightly higher than that of Ighwela *et al.* (2011) and slightly less than that of Anani and Nunoo (2016). The b-values obtained in the present study falls within the b-value range of 2-4, recommended for freshwater fish (Golam Mortuza & Al-Misned, 2013; Anani & Nunoo, 2016).

In the present study the condition factor (K) calculated for each treatment with their respective b-values, presented in Table 3.5, were significantly different from each other ($p<0.05$). These condition factors, when compared to condition factors obtained using Fulton's assumption of b = 3, were not significantly different ($p>0.05$). This is an indication that the growth of the fish in the current study do not deviate significantly from isometric growth. Moreover, according to Ighwela

et al. (2011), Ayoade (2011) and Anani & Nunoo (2016), a condition factor greater that one (K > 1) indicates good fish health and can also be an indication of isometric growth. The average K values *O. niloticus* in this study were all greater than one and greater than what Ighwela *et al.* (2011) reported for their *O. niloticus* in their study. The condition factor data, presented in Table 3.4, indicates that as CBD concentration increases across treatments that condition factors decreases.

3.8. Conclusion

The different CBD inclusion levels did not affect the growth of *O. niloticus* in this study.

The b-values for the treatments differed significantly. Although the observed b-values were slightly lower than that of the assumption created by the Fulton's condition index of b = 3, the acceptable range of 2-4 recommended by Golam Mortuza & Al-Misned (2013) and Anani & Nunoo (2016). This increasing b-value trend observed across increasing CBD inclusion level could suggest that at some stage fish could cross the isometric growth stage into the positive allometric growth phase which would mean that fish grow faster in relation to their length and can be a possible area of focus for future studies.

The body condition factor (K) in the present study were significantly different between treatments. Furthermore, the K-values obtained with the calculated b-values and that assumed by Fulton's condition index, were not significantly different from each other. This suggests that although the b-values indicate a deviation from isometric growth that it in actual fact can be accepted that the fish in the present study experienced isometric growth (Ighwela *et al.*, 2011; Ayoade, 2011; Anani & Nunoo, 2016). Moreover, the observed K-values are well above the minimum K-value recommended for Nile Tilapia by Ayoade (2011) which is an indication that the CBD did not affect their feed intake, and overall wellbeing.

References

Abdelhadi, Y. M. 2011. Tilapia: From the Nile to the World. J. Agric. Sci. Technol. 5, 251–255.

Al-Ghanim, K., Al-Thobaiti, A., Al-Balawi, H. F. A., Ahmed, Z., & Mahboob, S. 2017. Effects of replacement of fishmeal with other alternative plant sources in the feed on proximate composition of Muscle, Liver and Ovary in Tilapia (Oreochromis nioloticus). Brazilian Arch. Biol. Technol. 60, 1–7 https://doi.org/10.1590/1678-4324-2017160376.

Anani, F. ., & Nunoo, F. K. . 2016. Length-weight relationship and condition factor of Nile tilapia , Oreochromis niloticus fed farm-made and commercial tilapia diet. Int. J. Fish. Aquat. Stud. 4, 647–650.

Atakan, Z. 2012. Cannabis, a complex plant: Different compounds and different effects on individuals. Ther. Adv. Psychopharmacol. 2, 241–254 https://doi.org/10.1177/2045125312457586.

AVI Feeds. 2020. Commercial Fish.

Ayoade, A. A. 2011. Length-weight Relationship and Diet of African Carp Labeo ogunensis (Boulenger, 1910) in Asejire Lake Southwestern Nigeria. J. Fish. Aquat. Sci. 6, 472–478 https://doi.org/10.3923/jfas.2011.472.478.

Barnham, C., & Baxter, A. 1998. Condition Factor, K, for Salmonid Fish.

Di Marzo, V. 2008. Targeting the endocannabinoid system: to enhance or reduce? Nat. Rev. Drug Discov. 7, 438–455 https://doi.org/10.1038/nrd2553.

Di Marzo, V., & Matias, I. 2005. Endocannabinoid control of food intake and energy balance. Nat. Neurosci. 8, 585–589 https://doi.org/10.1038/nn1457.

Djissou, A. S. M., Adjahouinou, D. C., Koshio, S., & Fiogbe, E. D. 2016. Complete replacement of fish meal by other animal protein sources on growth performance of Clarias gariepinus fingerlings. Int. Aquat. Res. 8, 333–341 https://doi.org/10.1007/s40071-016-0146-x.

Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Busca, K., Egan, F., Muller, N., Whooley, J., McGinnity, P., Culloty, S., Ross, R. P., & Stanton, C. 2020. Replacing fishmeal with plant protein in Atlantic salmon (Salmo salar) diets by supplementation with fish protein hydrolysate. Sci. Rep. 10, 1–16 https://doi.org/10.1038/s41598-020-60325-7.

El-Sayed, A. F. M. 2006. Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials. Av. en Nutr. Acuícola VIII, 95–106.

El-Shafai, S. A., El-Gohary, F. A., Nasr, F. A., Van Der Steen, N. P., & Gijzen, H. J. 2004. Chronic ammonia toxicity to duckweed-fed tilapia (Oreochromis niloticus). Aquaculture 232, 117–127 https://doi.org/10.1016/S0044-8486(03)00516-7.

Food and Agriculture Organization of the United Nations. 2020. The State of World fisheries and aquaculture in review. FAO.org, Rome.

Food and Agriculture Organization of the United Nations (FAO). 2016. Oreochromis niloticus (Linnaeus, 1758). Rome.

Froese, R. 2006. Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22, 241–253 https://doi.org/https://doi.org/10.1111/j.1439-0426.2006.00805.x.

Fry, J. P., Mailloux, N. A., Love, D. C., Milli, M. C., & Cao, L. 2018. Feed conversion efficiency in aquaculture: Do we measure it correctly? Environ. Res. Lett. 13 https://doi.org/10.1088/1748-9326/aaa273.

Golam Mortuza, M., & Al-Misned, F. A. 2013. Length-Weight Relationships, Condition Factor and Sex-Ratio of Nile Tilapia, Oreochromis niloticus in Wadi Hanifah, Riyadh, Saudi Arabia. World J. Zool. 8, 106–109 https://doi.org/10.5829/idosi.wjz.2013.8.1.7247.

Gomiero, L. M., & De Souza Braga, F. M. 2005. The condition factor of fishes from two river basins in São Paulo state, Southeast of Brazil. Acta Sci. - Biol. Sci. 27, 73–78 https://doi.org/10.4025/actascibiolsci.v27i1.1368.

Hossain, M. Y., Ahmed, Z. F., Leunda, P. M., Jasmine, S., Oscoz, J., Miranda, R., & Ohtomi, J. 2006. Condition, length-weight and length-length relationships of the Asian striped catfish Mystus vittatus (Bloch, 1794) (Siluriformes: Bagridae) in the Mathabhanga River, southwestern Bangladesh. J. Appl. Ichthyol. 22, 304–307 https://doi.org/10.1111/j.1439-0426.2006.00803.x.

Ighwela, K. A., Ahmed, A. B., & Abol-Munafi, A. B. 2011. Condition Factor as an Indicator of Growth and Feeding Intensity of Nile Tilapia Fingerlings (Oreochromis niloticus) Feed on Different Levels of Maltose. Am. J. Agric. Environ. Sci 11, 559–563.

Ignatowska-Jankowska, B., Jankowski, M. M., & Swiergiel, A. H. 2011. Cannabidiol decreases body weight gain in rats: Involvement of CB2 receptors. Neurosci. Lett. 490, 82–84 https://doi.org/10.1016/j.neulet.2010.12.031.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. 2009. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol. Sci. 30, 515–527 https://doi.org/10.1016/j.tips.2009.07.006.

Jones, R. E., Petrell, R. J., & Pauly, D. 1999. Using modified length-weight relationships to assess the condition of fish. Aquac. Eng. 20, 261–276 https://doi.org/10.1016/S0144-8609(99)00020-5.

Joscelyne, M. 2020. The influence of Aquahatch on the growth performance of larval and juvenile African catfish (Clarias gariepinus , Burchell 1822).

Khallaf, E. A., Galal, M., & Authman, M. 2003. The biology of Oreochromis niloticus in a polluted canal. Ecotoxicology 12, 405–416 https://doi.org/10.1023/a:1026156222685.

Kleinhenz, M. D., Magnin, G., Ensley, S. M., Griffin, J. J., Goeser, J., Lynch, E., & Coetzee, J. F. 2020. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. Appl. Anim. Sci. 36, 489–494 https://doi.org/10.15232/aas.2020-02018.

Le Cren, E. D. 1951. The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in the Perch (Perca fluviatilis). J. Anim. Ecol. 20, 201–219 https://doi.org/10.2307/1540.

Luckhoff, P. D. 2005. Application of the condition factor in the production of African Sharptooth Catfish Clarias gariepinus.

Madage, S. S. K., Medis, W. U. D., & Sultanbawa, Y. 2015. Fish Silage as Replacement of Fishmeal in Red Tilapia Feeds. J. Appl. Aquac. 27, 95–106 https://doi.org/10.1080/10454438.2015.1005483.

Matias, I., Bisogno, T., & Di Marzo, V. 2006. Endogenous cannabinoids in the brain and peripheral tissues: regulation of their levels and control of food intake. Int. J. Obes. 30, S7–S12 https://doi.org/10.1038/sj.ijo.0803271.

Mohammadi, M., Imani, A., Farhangi, M., Gharaei, A., & Hafezieh, M. 2020. Replacement of fishmeal with processed canola meal in diets for juvenile Nile tilapia (Oreochromis niloticus): Growth performance, mucosal innate immunity, hepatic oxidative status, liver and intestine histology. Aquaculture 518, 734824 https://doi.org/10.1016/j.aquaculture.2019.734824.

Muchlisin, Z. A., Musman, M., & Siti Azizah, M. N. 2010. Length-weight relationships and condition factors of two threatened fishes, Rasbora tawarensis and Poropuntius tawarensis, endemic to Lake Laut Tawar, Aceh Province, Indonesia. J. Appl. Ichthyol. 26, 949–953 https://doi.org/10.1111/j.1439-0426.2010.01524.x.

Nash, R. D. M., Valencia, A. H., & Geffen, A. J. 2006. The origin of Fulton's condition factor - Setting the record straight. Fisheries 31, 236–238.

Omogoriola, H., Williams, A., Adegbile, O., Olakolu, F., Ukaonu, S., & Myade, E. 2011. Length- weight relationships, condition factor (K) and relative condition factor (Kn) of Sparids, Dentex congoensis (Maul, 1954) and Dentex angolensis (Maul and Poll, 1953), in Nigerian coastal water. Int. J. Biol. Chem. Sci. 5 https://doi.org/10.4314/ijbcs.v5i2.72147.

Pauly, D. 1984. Fish population dynamics in tropical waters: A manual for use with programmable calculators. 143rd ed. International Center for Living Aquatic Resources Management, Manila, Philippines.

Pertwee, R. G. 2004. The pharmacology and therapeutic potential of cannabidiol.Pages 32–83 in Cannabinoids. Di Marzo, V., ed. Kluwer Academic/Plenum Publishers, New York.

Pertwee, R. G. 2009. Emerging strategies for exploiting cannabinoid receptor agonists as medicines. Br. J. Pharmacol. 156, 397–411 https://doi.org/10.1111/j.1476-5381.2008.00048.x.

R Core Team. 2020. R: A Language and Environment for Statistical computing.

Ricker, W. E. 1975. Computation and Interpretation of Biological Statistics of Fish Populations (JC Stevensons, J Watson, RH Wigmore, & JM Reinhart, Eds.). Department of the Environment, Fisheries and Marine Service, Ottawa.

Riedel, G., Fadda, P., McKillop-Smith, S., Pertwee, R. G., Platt, B., & Robinson, L. 2009. Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. Br. J. Pharmacol. 156, 1154–1166 https://doi.org/10.1111/j.1476-5381.2008.00107.x.

Ross, L. G. 2000. Environmental physiology and energetics.Pages 89–128 in Tilapias: Biology and Exploitation. Fish and Fisheries Series, vol 25. Beveridge, M.C.M., McAndrew, B.J., eds. Fish & Fisheries Series. Springer Netherlands, Dordrecht.

Russo, E. B. 2007. History of Cannabis and Its Preparations in Saga, Science, and Sobriquet. Chem. Biodivers. 4, 1614–1648 https://doi.org/10.1002/cbdv.200790144.

Saoud, I., Babikian, J., Nasser, N., & Monzer, S. 2018. Effect of cannabis oil on growth performance, haematology and metabolism of Nile Tilapia Oreochromis niloticus. Aquac. Res. 49, 809–815 https://doi.org/10.1111/are.13512.

Shi, C., Liu, Y., Yi, M., Zheng, J., Tian, H., Du, Y., Li, X., & Sun, G. 2017. Comparison of time-restricted and ad libitum self-feeding on the growth, feeding behavior and daily digestive enzyme profiles of Atlantic salmon. Chinese J. Oceanol. Limnol. 35, 729–736 https://doi.org/10.1007/s00343-017-5346-8.

Tacon, A. G. J., & Metian, M. 2015. Feed Matters: Satisfying the Feed Demand of Aquaculture. Rev. Fish. Sci. \& Aquac. 23, 1–10 https://doi.org/10.1080/23308249.2014.987209.

Tarkan, A. S., Gaygusuz, O., Acipinar, H., Gursoy, C., & Ozulug, M. 2006. Length-weight relationship of fishes from the Marmara region (NW-Turkey). J. Appl. Ichthyol. 22, 271–273 https://doi.org/10.1111/j.1439-0426.2006.00711.x.

Wang, M., Liang, C., Hu, H., Zhou, L., Xu, B., Wang, X., Han, Y., Nie, Y., Jia, S., Liang, J., & Wu, K. 2016. Intraperitoneal injection (IP), Intravenous injection (IV) or anal injection (AI)? Best way for mesenchymal stem cells transplantation for colitis. Sci. Rep. 6, 1–13 https://doi.org/10.1038/srep30696.

Wiley, J. L., Burston, J. J., Leggett, D. C., Alekseeva, O. O., Razdan, R. K., Mahadevan, A., & Martin, B. R. 2005. CB 1 cannabinoid receptor-mediated modulation of food intake in mice. Br. J. Pharmacol. 145, 293–300 https://doi.org/10.1038/sj.bjp.0706157.

Zar, J. H. 1984. Biostatistical Analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs.

Zuardi, A. W. 2008. Cannabidiol : from an inactive cannabinoid to a drug with wide spectrum of action Canabidiol : de um canabinóide inativo a uma droga com amplo espectro de ação. Rev. Bras. Psiquiatr. 30, 271–280 https://doi.org/10.1590/S1516-44462008000300015.

Chapter 4 Effects of CBD oil extract on WBC, RBC and Platelets

4.1. Abstract

In this study, CBD oil as a feed additive was studied to investigate its effects on haematological parameters (WBC, RBC and Platelets) and to determine its potential effects on immunology of Nile Tilapia. Four treatments were prepared with increasing CBD concentrations (0 mg/kg, 20 mg/kg, 40 mg/kg, and 60 mg/kg). The treatments were offered to Nile Tilapia for a duration of 10 weeks. Blood samples was taken bi-weekly and assessed by means of flow cytometry (BD FACSMelody – Data File Structure: Flow Cytometry Standard (FCS) 3.0 and 3.1). The results indicated that CBD had no effect on blood cell counts.

4.2. Introduction

Aquaculture is the fastest-growing food production sector in the world (FAO, 2020). Recent global aquaculture production data as of 2018 indicate that world aquaculture production was an all-time record high of 114.5 million tonnes live weight, valued at USD 263.6 billion (FAO, 2020). The African contribution according to Halwart (2020) stands at 2.7%, notwithstanding according to Cai *at al.* (2017) and FAO (2018), this contribution will increase significantly as large scale investments are taking place across the continent which will enable the continent to produce large quantities of fish. South Africa is the tenth country of the top 10 aquaculture producers of Africa with a regional contribution of 0.28% and a global share of 0.01% (Adeleke *et al.*, 2021). Halley and Semoli (2021) reports that the South African marine aquaculture grew with 407 tonnes and 913 tonnes from 2017 – 2018 and 2018 – 2019, respectively. Furthermore, freshwater aquaculture grew with 293 tonnes and 95 tonnes from 2017 – 2018 and 2018 – 2019, respectively (Halley & Semoli, 2021).

Taking into consideration the growth that existed for 2017 – 2019, together with historic data (Halley & Semoli, 2021), one can expect growth as soon as the Covid19 pandemic can be managed and the world returning to its new normal. This potential future growth then is indicative of more intensive production systems to make production goals since Cai *et al.* (2017) and FAO (2018) predict substantial investments to take place in Africa.

According to Cnaani *et al.* (Cnaani, 2006), fish in intensive production systems are in an extended stressful environment, together with high stocking densities causing deterioration in water quality (Saoud *et al.*, 2018), leading to disease outbreaks causing mass mortalities (William & Do-hyung,

2015; Adeleke *et al.*, 2021). Furthermore, disease outbreaks place limitations on aquaculture production that have substantial financial implications that threaten the economic viability of the sector (Fazio, 2019).

The Endocannabinoid system is complex and widely distributed throughout the body for its essential role in modulatory functions like immune status, inflammation, and emotional response. Cannabidiol (CBD) is one of 150 phytocannabinoids found within resin within flowers, leaves, and seeds of *Cannabis sativa* (Atakan, 2012; Shahbazi *et al.*, 2020), and is the second most studied cannabinoid after delta-9-tetrahydrocannabinol (THC). According to Costa *et al.* (2007), CBD has an immunomodulatory effect that stimulates the immune system to fight disease. Furthermore, CBD also has an anxiolytic effect on humans that is known for relieving anxiety (Crippa *et al.*, 2009). This might be beneficial in intensive production systems and occurrences of diseases as a result of overcrowding, might be lowered.

CBD are considered to be an antagonist with low binding affinity at the orthostatic site at the CB1 receptor and an agonist at the CB2 receptor (McPartland *et al.*, 2015; Hazzah *et al.*, 2020; Shahbazi *et al.*, 2020). The specific mechanisms of action for biological effects caused by CBD remains unclear (Hazzah *et al.*, 2020; Gray & Whalley, 2020) because at least 65 molecular targets for CBD exists and its ”activity is multimodal” with the vast majority of it not being cannabinoid receptor dependent (Ibeas Bih *et al.*, 2015; Hazzah *et al.*, 2020).The effects of CBD on the immune response of fish have not yet been published and therefore needs to be elucidated.

A feed additive that can be added to any fish feed to improve the wellbeing and health of fish would not just be opportune for the industry but also to subsistence farmers in Africa, promoting the following Sustainable Development Goals (SDGs):

- SDG 1 – “End extreme poverty in all forms by 2030.”

- SDG 2 – “End hunger, achieve food security and improved nutrition and promote sustainable agriculture”

- SDG 8 – “Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all” (United Nations Development Programme, 2015b).

A feed additive that is natural and can improve the health and wellbeing of fish, can promote SDG 2 and 8 by being able to produce more fish under higher stocking densities to achieve food

security in villages, communities, and cities around the world and, providing existing aquaculture focused companies the ability to experience sustainable economic growth with lowered occurrences of loss of stock as a result of disease, respectively.

After carps, Nile Tilapia *(O. niloticus)* is the second most cultured fish in the world (Azaza *et al.*, 2008; Abdelhadi, 2011; Saoud *et al.*, 2018). Nile Tilapia are increasingly being cultured as its popularity increases globally. Although Nile Tilapia have a tendency to be more resistant than other farmed fish for “viral, bacterial, and parasitic diseases” (Weinert *et al.*, 2015), this study investigated the effects of CBD on haematological parameters for insight into potential immunology effects.

4.3. Materials and Methods

Ethical approval for the study was obtained from the Research Ethics Committee: Animal Care and Use (ACU-2020-14573).

4.3.1. Experiment location

All studies relating to the growth experiment were performed at the facilities of the Aquaculture Division of Stellenbosch University on the Welgevallen Experimental Farm. The experimental recirculating aquaculture system (RAS) was situated in one of the plastic growth tunnels of the Agronomy Department.

4.3.2. Experimental animals and Husbandry

Nile Tilapia (*Oreochrmis niloticus*) were used as experimental fish in this study. The stocking density were 30 fish per tank and six tanks per treatment, connected to a biological filter also acting as the header tank, a three-tank settling mechanical filter as well as a two-tank sump. Each treatment had 180 fish and in total 720 fish were used. Fish were reared in 28 circular fibre glass (D = 150 cm; H = 30 cm) tanks with the volume capacity of 350 l each. Fish were fed a commercial Tilapia diet at a feeding regime recommended by the manufacturer, 5% body weight at three times per day.

Water in the system was aerated using a regenerative blower and submerged 30 mm air stones. The RAS-setup was in an agricultural plastic growth tunnel that is influenced by high and low temperatures for the day. However, temperature ranged between 25 - 34 ˚C on average at feeding times. Temperature, pH, and dissolved oxygen were measured before feeding occurred. ammonia, nitrite, and nitrate nitrogen were measured bi-weekly.

4.3.3. Experimental design and treatments

At the start of the experimental trial, Tilapia of similar sizes was placed in the 24 tanks. The fish were offered; a commercial feed with 45% crude protein and 8.5% fat at 5% total average body weight (BW) three times a day, a 40% crude protein, and an 8.6% fat commercial feed at 5% total average BW three times a day, and a 35% crude protein and 7.4% fat commercial feed at 5% total average BW three times a day (AVI Products (Pty) Ltd., Reg. No. 2001/015923/07, Cato Ridge, Kwazulu-Natal, South Africa).

The control and three treatments (T20, T40 and T60) were assigned according to each of the four rows of tanks, illustrated in

Figure **3.1**. Each of the three treatments and control group were assigned to 6 of the 24 tanks, each. The Tilapia in treatments 20, 40 and 60 were offered a prepared diet and one unprepared diet functioning as the control. The prepared diet was fed at 5% BW of the average fish weight per tank in each of the treatments at 10:00, 13:00 and 16:00. The fish will be offered the above-mentioned diets for 10 weeks. Feeding started at 10 am as the water temperature before 10 am was below the optimum water temperature required. Literature indicates that feeding outside the optimum water temperature ranges leads to an increased FCR and contributes to bad water quality.

CBD isolates were derived from hemp (*Cannabis sativa L.)* mixed with industrial hemp seed oil extracted by means of cold press extraction. The oil was obtained from a commercial seller, Milagro. The product come in a range of different inclusion levels of CBD that is pre-determined, the highest CBD inclusion level product were chosen i.e., 2000 mg per 30 ml. The CBD extract were mixed with virgin coconut oil in a ratio of 1-part CBD oil to 500 parts coconut oil (1:500) i.e., 0.1 ml CBD oil for every 50 ml of virgin coconut oil per Kg of feed because the amount of CBD oil extract used for each treatment would not have been able to cover all pellets equally. The virgin coconut oil did not just serve as a medium to dilute the CBD concentration, but also one to ensure that the predetermined CBD concentration can be well covered over the pellets. The oil mixture was then used as a top cover for the Tilapia starter 2mm feed, Table 4.1 obtained from AVI Products (AVI Products (Pty) Ltd., Reg. No. 2001/015923/07, Cato Ridge, Kwazulu-Natal, South Africa).

The fish were fed a feed consisting of a floating pellet that ensured fish with increased appetite could feed on the uneaten feed and do not lose weight as result of increased metabolism as described by (Saoud *et al.*, 2018).

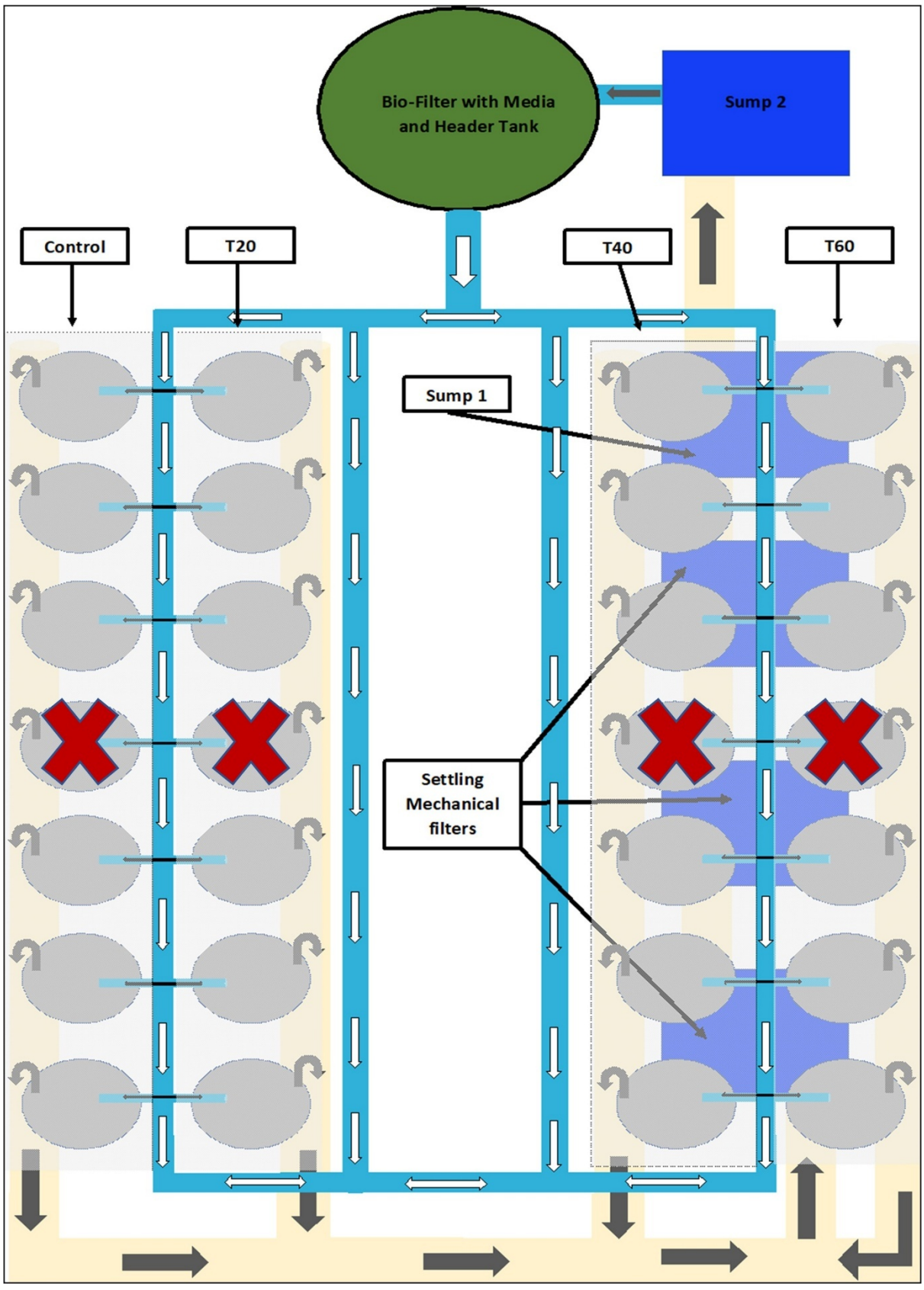


Figure 4.1 Schematic of system design (Jocelyn & Juries, 2020). Tanks marked with X, are fish selected from each treatment for haematology.

4.3.4. Experimental diet preparation

CBD-isolate were derived from hemp (*Cannabis sativa L.)* mixed with industrial hemp seed oil extracted by means of cold press extraction. The oil was obtained from a commercial seller, Milagro. The product come in a range of different inclusion levels of CBD that is pre-determined, the highest CBD inclusion level product were chosen i.e., 2000 mg per 30 ml. The CBD extract were mixed with virgin coconut oil in a ratio of 1-part CBD oil to 500 parts coconut oil (1:500) i.e., 0.1 ml CBD oil for every 50 ml of virgin coconut oil per Kg of feed because the amount of CBD oil extract used for each treatment would not have been able to cover all pellets equally. The virgin coconut oil did not just serve as a medium to dilute the CBD concentration, but also one to ensure that the predetermined CBD concentration can be well covered over the pellets. The oil mixture was then used as a top cover for the Tilapia starter 2mm feed, **Table 4.1** obtained from AVI Products (AVI Products (Pty) Ltd., Reg. No. 2001/015923/07, Cato Ridge, Kwazulu-Natal, South Africa).

From here onwards, T20, T40 and T60 refers to treatments and the numeric values refers to the CBD concentrations (20, 40, and 60 mg/kg) used in these treatments.

The T20 diet oil was prepared by mixing 9 ml of CBD oil (600 mg predetermined by the manufacturer) with 1 500 ml of virgin coconut oil. This oil mixture was then used as a top cover over 30 kg of feed. The oil was mixed using a Kenwood stand mixer at slow speed for about 10 minutes to ensure the coconut oil – CBD oil mixture was thoroughly mixed.

The T40 diet oil was prepared by mixing 18 ml of CBD oil extract (1200 mg predetermined by the manufacturer) with 3 000 ml of virgin coconut oil. This oil mixture was used as a top cover for 30 kg of feed. The same mixing and storage protocol followed in the preparation of diet T20 was followed.

The T60 diet oil was prepared by mixing 27 ml of CBD oil extract (1800 mg, predetermined by the manufacturer) with 3 600 ml of virgin coconut oil. This oil mixture was used as a top cover for 30 kg of feed. The same mixing and storage protocol as in T20 and T40 was followed.

A total of 90 kg of Tilapia grower 2mm feed was covered with the coconut oil – CBD mixture. To ensure that all pellets were evenly coated with the oil, batches of 10 kg was used at a time. A MacAdams commercial dough mixer was used to mix pellets with oil and to ensure all pellets were evenly covered with oil. The oil on the coated pellets was then allowed to solidify in a temperature-controlled room at 20˚C after which it was stored in the fish feed storage facility at

Welgevallen Experimental Farm. The same method was used when the control group's feed was covered with just coconut oil.

After the diet preparation of each treatment group was completed, all utensils and equipment used in the preparation was washed and wiped with 70% ethanol to ensure sterility and to prevent contamination.

Table 4.1 Feed formulation of Commercial Tilapia feed for each feed type adapted from AVI Feeds (2020).

Nutritional composition of Tilapia feeds

Nutrient		Tilapia Fry (No.0 powder and No.1,2,3 crumble)	Tilapia Starter 2mm	Tilapia Grower 3mm	Tilapia Finisher 5mm
Metabolisable Energy	MJ/kg	12|8	12|9	12|7	13|3
Digestible Energy	MJ/kg	14|8	14|9	14|7	14|8
Crude Protein	g/kg	450	400	350	300
Lysine	g/kg	25|2	21|4	18|6	15|3
Methionine	g/kg	9|5	7|5	6|1	5|6
T.S.A.A.	g/kg	16|9	14|3	12|4	11|5
Isoleucine	g/kg	19|3	17|3	15|4	13|7
Tryptophan	g/kg	4|3	4	3|7	3|1
Threonine	g/kg	18|6	16|2	14|1	12|8
Valine	g/kg	24|1	21	18|3	16|8
Arginine	g/kg	28|7	26|2	23|4	21|2
Fat	g/kg	85	86	74	77
Fibre	g/kg	23	29	32	28
Ash	g/kg	93	75	62	55
Calcium	g/kg	15|7	12|8	10|6	10|2
Total Phosphorus	g/kg	12|4	11|6	9|4	8|8
Sodium	g/kg	3|2	2|6	2|4	2

Sizes of the Crumbles	
#0	<500µm
#1	500-750µm
#2	750-1500µm
#3	1500-2500µm

4.3.5. Blood sampling

Blood was drawn every fortnight from 20 fish. Five fish per treatment were selected for haematological analysis. Fish were anaesthetized with clove oil at about 0.1 mL/L to reduce handling stress (da Silva *et al.*, 2021). Fish were placed on a damp cloth to keep them moist throughout the procedure. The head of each fish was covered with parts of the damp cloth to further reduce stress.

Blood was drawn from fish using the caudal venous puncture method as described by the Canadian Department of Fisheries and Oceans (2004). Between 0.1 - 0.5 mL of blood was drawn per fish with a 22-gauge needle using a 5 mL syringe and transferred to a flow cytometry tube containing 50 units of heparin. Weinert *et al.* (2015) used 50 IU per 0.8 - 1.5 mL blood sample. The fish were then placed back into their designated tanks according to treatments, tanks marked with red “X’s” (Figure 4.1).

The blood samples were then placed in a Styrofoam cooler with ice bricks for transport to the Central Analytical Facility (CAF) about 10 minutes from the Experimental site.

4.3.6. Sample analysis

The blood samples were prepared for analysis by creating samples containing a 1:3 ratio of blood to Phosphate Buffered Saline (PBS). Blood was pipetted through a fine nylon mash filter and 300 µL of the remaining blood sample were added to 600 µL of PBS to dilute the sample. Dilution of blood samples were required as the blood were clotting in the Fluorescence-activated cell sorting (FACS) flow cytometer (BD FACSMelody – Data File Structure: Flow Cytometry Standard (FCS) 3.0 and 3.1).

After successful cell sorting occurred, the data were analysed using BD FACSChorus software v1.1. About 50 000 cells were sampled that were used as the ‘parent sample’. From the parent sample, ‘gating’ a procedure in cell sorting used to distinguish cells from another, were used to identify platelets (thrombocytes), white blood cells (leukocytes) from here on referred to as WBC and red blood cells (erythrocytes) from here on referred to as RBC.

4.3.7. Statistical analysis

The latest version of R (R Core Team, 2020). (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/) was used where ANOVA was used to analyse haematological paramters together with test assumptions (normality and homoscedasticity) at p=0.05.

4.4. Results

Red blood cells (RBC), White blood cells (WBC), and Platelet counts per 50 000 cell sample of juvenile *O. niloticus* were subjected to a one-way ANOVA and did not differ significantly between treatments (P = 0.516, P = 0.994, and P = 0.652, respectively) (Table 4.2).

Table 4.2 The influence of CBD supplementation in Tilapia (Oreochromis niloticus) diets on the number (mean ± SE) of red blood cells (RBC), white blood cells (WBC), and platelets, respectively.

	CBD inclusion level			
Parameter	Control (n=30)	T20 (n=30)	T40 (n=30)	T60 (n=30)
Red blood cells ($X10^3$ per sample) ± SE	38.31 ± 0.67	36.97 ± 1.45	36.68 ± 1.14	38.42 ± 0.60
White blood cells ($X10^3$ per sample) ± SE	5.36 ± 0.56	5.41 ± 0.59	5.48 ± 0.55	5.58 ± 0.57
Platelets ($X10^3$ per sample) ± SE	5.53 ± 0.34	5.67 ± 0.4	6.05 ± 0.27	5.65 ± 0.24

4.5. Discussion

4.5.1. Effect of Cannabidiol (CBD) extract on haematological parameters

4.5.2. Effect of CBD on Red Blood Cell (RBC) count

One of the highly expressed proteins related to gas exchange in RBCs is haemoglobin (Shen *et al.*, 2018). Haemoglobin (Hb) is the ”iron-containing oxygen-transport metalloprotein” in RBCs (Shen *et al.*, 2018). According to Shen *et al.* (2018), the Reads per Kilobase of transcript per Million mapped reads (RPKM) value for haemoglobin (alpha and beta) was about 1.38 million, this accounted for more than 80% of expressed transcripts and indicates that RBC’s main function is oxygen delivery. Although studies by Lewis *et al.* (2010), Morera *et al.* (2011; 2011), and Shen *et al.* (2018) evidenced that RBCs from Nile Tilapia “expressed immune-related genes and responses”.

A decrease in RBC could mean that lowered immunity and an increase can infer better immunity. A decrease or increase in RBC also means that subsequently the Hb will also decrease and increase with RBC count. This can infer that that a lowered RBC not only could have an impact on the immunity of the fish, but also could have an effect on the amount of DO that the fish can take in. Overall, a lowered Hb level can mean a lowered availability of internal oxygen to cells used for energy for perfusion, ventilation, locomotion, and recovery after trauma (Kramer, 1987; Shultz *et al.*, 2011).

In the present study, a decrease in RBCs were noticed across treatments (), however it is not statistically significant.

4.5.3. Effect of CBD on Platelet count

In a study conducted by Elarabany *et al.* (2017) platelet counts was significantly higher in treatment groups with NaCl concentrations of 8 g/l and 12 g/l. According to Hosseinzadeh *et al.* (2013), Amin *et al.* (2016) and Elarabany *et al.* (2017), platelet count elevation may be caused by a non-specific immune response as a result of salinity stress, however, salinity stress could not have been the cause of the increase in platelet counts as all of the treatments were in the same RAS unit with the same water. This non-specific immune response could be an indication that platelets may increase in an immune response in order to facilitate recovery after a stressful event like high salinity. Stosik *et al.* (2002) found that platelets have the capacity to phagocytose the *Staphylocossus aureus* 209P strain and to destroy bacteria ingested by fish. Furthermore, Stosik *et al.* (2002) concludes that platelets act as a protective barrier.

In the present study, an increase in platelet counts were noticed across treatments; Control, T20, and T40, but a decrease for T60 (Table 4.2), however it is not statistically significant.

4.5.4. Effect of CBD on WBC count

Oseni *et al.*, (2006) reported no effect of cannabis on haematological values with marginally lower WBC count recorded for cannabis in human users than non-users. The study by Saoud *et al.* (2018) evidenced a similar lack of effect and together with the study by Oseni *et al.* (2006), attributed to the assertion by Hall and Solowji (1998) that cannabinoids reduce humoral and cell mediated immune responses by inhibiting B and T lymphocytes.

Looking at Figure 4.4, the present study evidenced contrasting effects for CBD. According to Silveira-Coffigny *et al.* (2004) and Martins *et al.* (2008), Nile Tilapia produced more white blood cells when it got infected with *Enterococcus* sp.. The WBC counts across the treatments (Table 4.2) increase with an increase in CBD concentration. If the treatments each had their own experimental unit it would have been easy to entertain the idea that an infection caused the increase in WBC, but because it is part of one system, an infection would not have caused differential WBC counts among treatments, seemingly responding to the treatments.

In the present study, an increase in WBC counts were noticed across treatments, however it is not statistically significant.

4.6. Conclusion

The present study solely focused on CBD and no other phytocannabinoids. This study presented findings that is in line with previous studies into the effects of *Cannabis sativa* on haematological parameters like RBC, WBC, and Platelet counts. Although there is a lack of studies on the effect of CBD on haematological parameters of fish, previous studies on the effect of *Cannabis sativa*, which contains CBD, showed similar results. This is an indication that although there aren't significant results, it also is an indication that fish wellbeing was acceptable.

Future studies therefore should expand on this knowledge to fully understand the variable effects of phytocannabinoids, and other potential effects not studied in the study.

References

Abdelhadi, Y. M. 2011. Tilapia: From the Nile to the World. J. Agric. Sci. Technol. 5, 251–255.

Adeleke, B., Robertson-Andersson, D., Moodley, G., & Taylor, S. 2021. Aquaculture in Africa: A Comparative Review of Egypt, Nigeria, and Uganda Vis-À-Vis South Africa. Rev. Fish. Sci. Aquac. 29, 167–197 https://doi.org/10.1080/23308249.2020.1795615.

Amin, F. B., Farhana, T., Mostakim, G. M., Zahangir, M. M., Mishu, M. M., & Islam, M. S. 2016. Behavioral and physiological stress responses of Java barb (Barbonymus gonionotus) to environmental salinity challenge. J. Aquac. Eng. Fish. Res. 2, 176–184 https://doi.org/10.3153/jaefr16019.

Atakan, Z. 2012. Cannabis, a complex plant: Different compounds and different effects on individuals. Ther. Adv. Psychopharmacol. 2, 241–254 https://doi.org/10.1177/2045125312457586.

AVI Feeds. 2020. Commercial Fish.

Azaza, M., Dhraïef, M., Biology, M. K.-J. of thermal, & 2008, U. 2008. Effects of water temperature on growth and sex ratio of juvenile Nile tilapia Oreochromis niloticus (Linnaeus) reared in geothermal waters in southern Tunisia. J. Therm. Biol. 33, 98–105.

Cai, J., Quagrainie, K., & Hishamunda, N. 2017. Social and economic performance of tilapia farming in Africa (J Cai, K Quagrainie, & N Hishamunda, Eds.). Food and Agriculture Organization of the United Nations, Rome.

Canada Department of Fisheries and Oceans. 2004. Blood Sampling of Finfish.

Cnaani, A. 2006. Genetic perspective on stress response and disease resistance in aquaculture. Isr. J. Aquac. - Bamidgeh 58, 375–383 https://doi.org/10.46989/001c.20448.

Costa, B., Trovato, A. E., Comelli, F., Giagnoni, G., & Colleoni, M. 2007. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. Eur. J. Pharmacol. 556, 75–83 https://doi.org/10.1016/j.ejphar.2006.11.006.

Crippa, J. A., Zuardi, A. W., Martín-Santos, R., Bhattacharyya, S., Atakan, Z., McGuire, P., & Fusar-Poli, P. 2009. Cannabis and anxiety: a critical review of the evidence. Hum. Psychopharmacol. Clin. Exp. 24, 515–523 https://doi.org/https://doi.org/10.1002/hup.1048.

Elarabany, N., Bahnasawy, M., Edrees, G., & Alkazagli, R. 2017. Effects of Salinity on Some Haematological and Biochemical Parameters in Nile Tilapia, <i>Oreochromus niloticus</i> Agric. For. Fish. 6, 200 https://doi.org/10.11648/j.aff.20170606.13.

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action.

Fazio, F. 2019. Fish hematology analysis as an important tool of aquaculture: A review. Aquaculture 500, 237–242 https://doi.org/10.1016/j.aquaculture.2018.10.030.

Food and Agriculture Organization of the United Nations (FAO). 2018. The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. Licence: CC BY-NC-SA 3.0 IGO., Rome.

Food and Agriculture Organization of the United Nations (FAO). 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action.

Gray, R. A., & Whalley, B. J. 2020. The proposed mechanisms of action of CBD in epilepsy. Epileptic Disord. 22, 10–15 https://doi.org/10.1684/epd.2020.1135.

Hall, W., & Solowij, N. 1998. Adverse effects of cannabis. Lancet 352, 1611–1616.

Halley, K., & Semoli, B. 2021. National Aquaculture Sector Overview - South Africa. Rome.

Halwart, M. 2020. FAO Aquaculture Newsletter. Rome.

Hazzah, T., Andre, C., Richter, G., & Mcgrath, S. 2020. Cannabis in Veterinary Medicine: A Critical Review. AHVMA J. 61, 17–41.

Hosseinzadeh, S. H., Masaeli, S., Alizadeh, M., Negarestan, H., & Naji, T. 2013. A study on growth parameters, blood factors and proximate composition of rainbow trout (Oncorhynchus mykiss) cultured in underground brackish and freshwater. Iran. J. Fish. Sci. 12, 836–842 https://doi.org/10.22092/ijfs.2018.114323.

Ibeas Bih, C., Chen, T., Nunn, A. V. W., Bazelot, M., Dallas, M., & Whalley, B. J. 2015. Molecular Targets of Cannabidiol in Neurological Disorders. Neurotherapeutics 12, 699–730 https://doi.org/10.1007/s13311-015-0377-3.

Kramer, D. L. 1987. Dissolved oxygen and fish behavior. Environ. Biol. Fishes 18, 81–92 https://doi.org/10.1007/BF00002597.

Lewis, J. M., Hori, T. S., Rise, M. L., Walsh, P. J., & Currie, S. 2010. Transcriptome responses to heat stress in the nucleated red blood cells of the rainbow trout (Oncorhynchus mykiss). Physiol. Genomics 42, 361–373 https://doi.org/10.1152/physiolgenomics.00067.2010.

Martins, ML.a*, Mouriño, JLP.a, b, Amaral, GV.a, Vieira, FN.b, Dotta, G.a, Jatobá, AMB.a, B., & Pedrotti, FS.a, b, Jerônimo, GT.a, Buglione-Neto, CC.b and Pereira-Jr., G. . 2008. Haematological changes in Nile tilapia experimentally infected with Enterococcus sp . Alterações hematológicas em tilápia do Nilo infectada. Braz. J. Biol., 68, 657–661.

McPartland, J. M., Duncan, M., Di Marzo, V., & Pertwee, R. G. 2015. Are cannabidiol and Δ9-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br. J. Pharmacol. 172, 737–753 https://doi.org/10.1111/bph.12944.

Morera, D., & MacKenzie, S. A. 2011. Is there a direct role for erythrocytes in the immune response? Vet. Res. 42, 89 https://doi.org/10.1186/1297-9716-42-89.

Morera, D., Roher, N., Ribas, L., Balasch, J. C., Doñate, C., Callol, A., Boltaña, S., Roberts, S., Goetz, G., Goetz, F. W., & MacKenzie, S. A. 2011. Rna-seq reveals an integrated immune response in nucleated erythrocytes. PLoS One 6 https://doi.org/10.1371/journal.pone.0026998.

Oseni, B. S., Togun, V. A., & Taiwo, O. F. 2006. Effect of Marijuana Smoking on Some Hematological Parameters of Smokers. World J. Med. Sci. 1, 82–85.

R Core Team. 2020. R: A Language and Environment for Statistical computing.

Saoud, I., Babikian, J., Nasser, N., & Monzer, S. 2018. Effect of cannabis oil on growth performance, haematology and metabolism of Nile Tilapia Oreochromis niloticus. Aquac. Res. 49, 809–815 https://doi.org/10.1111/are.13512.

Shahbazi, F., Grandi, V., Banerjee, A., & Trant, J. F. 2020. Cannabinoids and Cannabinoid Receptors: The Story so Far. iScience 23, 101301 https://doi.org/10.1016/j.isci.2020.101301.

Shen, Y., Wang, D., Zhao, J., & Chen, X. 2018. Fish red blood cells express immune genes and responses. Aquac. Fish. 3, 14–21 https://doi.org/10.1016/j.aaf.2018.01.001.

Shultz, A. D., Murchie, K. J., Griffith, C., Cooke, S. J., Danylchuk, A. J., Goldberg, T. L., & Suski, C. D. 2011. Impacts of dissolved oxygen on the behavior and physiology of bonefish: Implications for live-

release angling tournaments. J. Exp. Mar. Bio. Ecol. 402, 19–26 https://doi.org/10.1016/j.jembe.2011.03.009.

da Silva, D. R., Arvigo, A. L., Giaquinto, P. C., Delicio, H. C., Barcellos, L. J. G., & Barreto, R. E. 2021. Effects of clove oil on behavioral reactivity and motivation in Nile tilapia. Aquaculture 532, 736045 https://doi.org/10.1016/j.aquaculture.2020.736045.

Silveira-Coffigny, R., Prieto-Trujillo, A., & Ascencio-Valle, F. 2004. Effects of different stressors in haematological variables in cultured Oreochromis aureus S. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 139, 245–250 https://doi.org/10.1016/j.cca.2004.11.009.

Stosik, M., Deptuła, W., Trávniček, M., & Baldy-Chudzik, K. 2002. Phagocytic and bactericidal activity of blood thrombocytes in carps (Cyprinus carpio). Vet. Med. (Praha). 47, 21–25 https://doi.org/10.17221/5798-VETMED.

United Nations Development Programme. 2015. Sustainable Development Goals.

Weinert, N. C., Volpato, J., Costa, Á., Antunes, R. R., De Oliveira, A. C., Mattoso, C. R. S., & Saito, M. E. 2015. Hematology of Nile tilapia (Oreochromis niloticus) subjected to anesthesia and anticoagulation protocols. Semin. Agrar. 36, 4237–4250 https://doi.org/10.5433/1679-0359.2015v36n6Supl2p4237.

William, I., & Do-hyung, K. 2015. Improving biosecurity in the nascent aquaculture industry of Eastern Africa. Page 213

Chapter 5 General Conclusions and Recommendations

5.1. General Conclusions

The global demand for sustainable fish, promoting the blue economy, has been a promoter of the culture of fish species like Tilapia. The costs associated with cost-efficient and sustainable farming of Tilapia, generally necessitates a degree of commercialisation. Commercialisation results in higher stocking densities to produce the cheapest protein, however, with higher stocking densities and increased production to reach economies of scale, comes the prevalence of disease, and decreased wellbeing of fish.

Cannabis sativa is an herbaceous plant that comprises of a cannabinoid known as Cannabidiol (CBD) which possess most of the properties of delta-9-tetrahydrocannabinol (THC) except the psychoactive effect. Properties of Cannabis sativa extract are becoming more understood because of the beneficial properties it possesses. The abovementioned phytocannabinoids can only have an effect via the Endocannabinoid System. The Endocannabinoid system is complex and widely distributed throughout the body for its essential role in modulatory functions like immune status, inflammation, and emotional response. Cannabis sativa contains more than 150 phytocannabinoids which can be found in resin within flowers, leaves, and seeds. Cannabidiol (CBD) is but one of these cannabinoids and is the second most studied cannabinoid to delta-9-tetrahydrocannabinol (THC). CBD is an antagonist at the CB1 receptor and an agonist at the CB2 receptor which has an immunomodulatory effect that stimulates the immune system to fight disease as well as an anxiolytic effect in humans.

A commercial Tilapia feed was supplemented with three different concentrations of CBD; 20 mg/kg, 40 mg/kg and 60mg/kg and was fed to Tilapia for 10 weeks. Data including live weight, fish length were recorded fortnightly, and blood samples were taken from 5 sample fish per treatment for flow cytometry to determine the influence of the treatment diets on the wellbeing of the fish. At termination of the trial, survival, growth parameters, and feed conversion ratio (FCR) were assessed.

The water temperature for the duration of the trial was on average 26 – 28 ˚C and 30 – 34 ˚C for week 1 – 5 and week 6 – 10, respectively. These temperatures were in line with optimal temperatures reported for *O. niloticus*. The pH of the study ranged between 7.00 – 7.80 over the duration of the 10 – week experimental period. This pH range is within the acceptable optimal range for *O. niloticus*. The DO of the study averaged at 5.63 mg/l with an averaged min and max

DO value recorded at 3.86 mg/l and 7.30 mg/l, respectively. On average, except for individual exclusionary instances, the DO was above the minimum for optimum growth.

The b-values for the treatments (Control, T20, T40 and T60) were significantly different from each other and indicates an increase as the CBD inclusion levels increased from T20 to T60. Although the observed b-values were slightly lower than that of the assumption created by the Fulton's condition index of b = 3, the acceptable range of 2-4 for b-values are recommended my literature.

Subsequently, the condition factor (K) is dependent on the b-value and will be influenced by it. In the present study as b-values increased across treatments, K-values decreased across treatments. K-values in the present study were significantly different between treatments. Furthermore, the K-values obtained with the calculated b-values and that assumed by Fulton's condition index, were not significantly different from each other. This suggests that although the b-values indicate a deviation from isometric growth that it in actual fact can be accepted that the fish in the present study experienced isometric growth. Moreover, the observed K-values are well above the minimum K-value recommended for Nile Tilapia which is an indication that the fish in the present study was of good- health, well-being, and nutrition.

The study presented findings that is in line with previous studies into the effects of *Cannabis sativa* on haematological parameters like RBC, WBC, and Platelet counts. What set this study apart from what was already done, was the sole use of the phytocannabinoid, CBD. Previous studies looked at THC, CBD-THC or *Cannabis sativa* that contains more than 750 bioactive compounds and over 150 phytocannabinoids.

Studies that were conducted on cannabinoids found that it reduced humoral and mediated immune responses. Studies that were conducted on THC and CBD together, found that CBD had an antagonistic effect to the effects of THC, solely as a result of the binding at the CB1 and CB2 receptors.

Although preliminary data showed indications of increasing and decreasing trends across treatments, it however, was not significant enough to assume that CBD do indeed cause a decrease or increase in RBC, WBC, and Platelet count.

5.2. Recommendations

The present study took individual weights and lengths and focused on the means per tank of each treatment. For the haematological parameters, a sample group was randomly selected from each treatment to represent the haematological parameters of that treatment.

Future studies should focus on individual fish to track the effects of the treatments on the fish. This will allow for a smaller experimental system which will reduce the effects caused by water quality parameters like water temperature, dissolved oxygen, pH and biological wastes like uneaten feed and faeces that impact the ammonia and nitrate levels in the system. Mitigating the abovementioned will reduce the impact it can have on the growth and well-being of fish. Tilapia fry were used in the present study and results might differ when fingerlings or juveniles are used. The effects caused by the environment, or the water quality parameters might also not have detrimental effects that could potentially influence the growth and overall well-being of the fish as it grows into fingerlings and juveniles.

The inclusion levels in the present study were adapted from previous studies on *Cannabis sativa* and hemp. However, future studies can use the inclusion levels in the present study to test the extreme effect of higher inclusion levels.

The fish in the present study were not stressed intentionally to test the effects of the treatment on stress and other hormonal induced effects experienced by the fish. Thus, future studies can investigate the treatment effects on hormonally induced behaviour.

Furthermore, the treatment effects presented by the present study on Tilapia might be different on another fish species like catfish or trout and should therefore also be of consideration for future investigations.